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Rapid detection of platelet inhibition and dysfunction in traumatic brain injury: A prospective observational study

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BACKGROUND: Rapid platelet function testing is frequently used to determine platelet function in patients with traumatic intracranial hemorrhage (tICH). Accuracy and clinical significance of decreased platelet response detected by these tests is not well understood. We sought to determine whether VerifyNow and whole blood aggregometry (WBA) can detect poor platelet response and to elucidate its clinical significance for tICH patients.

METHODS: We prospectively enrolled patients with isolated tICH between 2018 and 2020. Demographics, medical history, injury characteristics, and patient outcomes were recorded. Platelet function was determined by VerifyNow and WBA testing at the time of arrival to the trauma bay and 6 hours later.

RESULTS: A total of 221 patients were enrolled, including 111 patients on no antiplatelet medication, 78 on aspirin, 6 on clopidogrel, and 26 on aspirin and clopidogrel. In the trauma bay, 29.7% and 67.7% of patients on no antiplatelet medication had poor platelet response on VerifyNow and WBA, respectively. Among patients on aspirin, 72.2% and 82.2% had platelet dysfunction on VerifyNow and WBA. Among patients on clopidogrel, 67.9% and 88.9% had platelet dysfunction on VerifyNow and WBA. Patients with nonresponsive platelets had similar in-hospital mortality (3 [3.0%] vs. 6 [6.3%], $p = 0.324$), tICH progression (26 [27.1%] vs. 24 [26.1%], $p = 0.877$), intensive care unit admission rates (34 [34.3%] vs. 38 [40.0%), $p = 0.415$), and length of stay (3 [interquartile range, 2–8] vs. 3.2 [interquartile range, 2–7], $p = 0.818$) to those with responsive platelets. Platelet transfusion did not improve platelet response or patient outcomes.

CONCLUSION: Rapid platelet function testing detects a highly prevalent poor platelet response among patients with tICH, irrespective of antiplatelet medication use. VerifyNow correlated fairly with whole blood aggregometry among patients with tICH and platelet responsiveness detectable by these tests did not correlate with clinical outcomes. In addition, our results suggest that platelet transfusion may not improve clinical outcomes in patients with tICH. (*J Trauma Acute Care Surg.* 2022;92: 167–176. Copyright © 2021 The Author (s). Published by Wolters Kluwer Health on behalf of the American Association for the Surgery of Trauma.)

LEVEL OF EVIDENCE: Diagnostic tests, level II.

KEY WORDS: Platelet function testing; platelet transfusion; intracranial hemorrhage; brain injuries; traumatic.

Antiplatelet medications, such as aspirin and clopidogrel, are frequently used for prevention in patients with cardiovascular disease. However, these medications can lead to increased bleeding in patients following traumatic injury. Use of antiplatelet medication may be an independent risk factor for the development of intracranial hemorrhage in patients presenting with blunt head injury.[1,2] It is, therefore, important to ascertain the history of antiplatelet medication use, because it may affect patient outcomes and guide clinical management. However, medication history is frequently unknown in patients who present with traumatic brain injury (TBI) or cannot be determined in a timely fashion. In addition, among patients who are known to take antiplatelet medication, the proportion of "nonresponders"—individuals who retain their platelet function even while on aspirin or clopidogrel—can range from 5.5% to 45%.[3]

In an effort to more accurately and reliably determine platelet function in TBI patients, point-of-care (POC) platelet function tests, such as VerifyNow (Instrumentation Laboratory, Bedford, MA), are commonly used.[4–6] These tests allow for rapid determination of platelet inhibition due to antiplatelet medications and can be used to guide management. Although developed and validated for use to monitor platelet responses of patients with cardiovascular disease on antiplatelet drugs,[7,8] VerifyNow has also been adopted for use in trauma patients.[5] Trauma and TBI, in particular, frequently lead to platelet dysfunction.[9–12] How this change in platelet function after injury may impact the ability of VerifyNow to detect antiplatelet medication and whether this POC assay can detect endogenous decreased platelet response after trauma are not known. Throughout the article, we describe platelets as "responsive" or "nonresponsive" with respect to their function in response to stimulation by an agonist.

The objectives of this prospective observational study were to (1) evaluate platelet response in patients with isolated traumatic intracranial hemorrhage (tICH), using VerifyNow; (2) to evaluate the diagnostic performance of VerifyNow, as compared with whole blood aggregometry (WBA); (3) to evaluate the impact of platelet and/or whole blood transfusion on platelet response, as determined using VerifyNow and WBA; and (4) to examine the impact of platelet responsiveness on clinical outcomes.

METHODS

Patient Population

Patients were prospectively enrolled at a Level I trauma center between May 21, 2018, and July 30, 2020, in a consecutive series. The study was conducted in accordance with the "Standards for Reporting Diagnostic accuracy studies" guidelines. Inclusion criteria were: trauma system activation (any level), patient 18 years or older, injury requiring head CT, head CT showed an intracranial hemorrhage, head Abbreviated Injury Scale (AIS) score of 2 or more and less than 2 for all other body regions (determined by the members of the trauma team at the time of enrollment). The study was conducted under waiver of consent as approved

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by the institutional review board at the University of Pittsburgh (STUDY19020056). Exclusion criteria were: patients younger than 18 years, pregnancy, presence of platelet dysfunction due to underlying comorbid conditions (Bernard-Soulier syndrome, Glanzmann thrombasthenia, Gray Platelet Syndrome, Delta Storage Pool Deficiency, von Willebrand disease), patients on anticoagulants (direct oral anticoagulants, such as apixaban, dabigatran, rivaroxaban, edoxaban, and vitamin K anticoagulants, such as warfarin, acenocoumarol, pheprocoumon, fluindione), and patients with moderate to severe injuries to other parts of the body (AIS score, >1). Demographic information, injury information (including time of injury), medical history, medication use, laboratory values, initial CT head findings, repeat CT head findings, blood product administration (platelet and whole blood transfusion), and operative interventions were recorded. Decision to transfuse platelets or whole blood was based clinical judgment and routine practice of trauma surgery or neurosurgery teams and occurred independent of platelet function testing performed for research.

Platelet Function Testing

Platelet function testing using VerifyNow and WBA was performed on all patients at the time of arrival to the trauma bay (0 hour) and within 1 hour after platelet transfusion, or at 6 hours if the patient did not receive platelet transfusion. Clinicians were blinded to these research laboratories but may have ordered platelet function testing separately as part of routine clinical practice outside of the study. All patients were tested using VerifyNow Aspirin Platelet Reactivity Test (VNA) (Instrumentation Laboratory, Bedford MA) to detect decreased platelet response due to aspirin and VerifyNow PRUTest Platelet Reactivity Test (VNP) (Instrumentation Laboratory, Bedford, MA) to detect decreased platelet response due to P2Y12 inhibitors. Per manufacturer's guidelines, platelets were considered nonresponsive due to aspirin if the Aspirin Reaction Unit (ARU) index was less than 550 on VNA. Platelets were considered nonresponsive due to clopidogrel if P2Y12 Reaction Unit (PRU) index was less than 220 on VNP. This cutoff was chosen based on existing literature.[13] Whole blood aggregometry (ChronoLog Corporation, Havertown, PA) was used to determine platelet function in response to 0.5 mM of arachidonic acid (AA) and 5 μM of adenosine diphosphate (ADP). Platelets were considered nonresponsive due to aspirin if WBA amplitude in response to AA was less than 6 Ohms.[14] Platelets were considered nonresponsive due to clopidogrel if WBA amplitude in response to ADP was less than 5 Ohms.[15–17] Results of VNA and VNP were available to the performers of WBA at the time of testing.

Clinical Outcomes

Mortality was defined as death while in-hospital. Progression of tICH was defined as either expansion of an existing tICH on repeat head CT or as appearance of an additional tICH on repeat head CT. Progression of intracranial hemorrhage was adjudicated by the attending radiologist as part of standard clinical care and in accordance with local guidelines. Admission to intensive care unit (ICU), length of ICU stay (in days), total hospital stay (in days), and discharge location were noted.

Statistical Analysis

The presence of poor platelet response in tICH patients (objective 1) was determined using descriptive statistics. Data of continuous variables are summarized as median and interquartile range (IQR). With consideration of relatively small sample size and multigroup comparisons, differences between any two groups were tested using the nonparametric Wilcoxon rank sum test. Differences between more than two groups were tested using the nonparametric Kruskal-Wallis test and followed by post hoc testing using Dunn's test. Categorical variables summarized using Fisher's exact test. Classification methods using 2×2 contingency tables were used to generate sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) to quantify the diagnostic performance of VNA and VNP (objective 2) at the time of admission. Whole blood aggregometry with AA was used as reference for VNA and WBA with ADP was used as reference for VNP. The internal consistency was checked using Cronbach's alpha and interrater reliability was tested using κ statistics. Univariate regression with multivariate adjustment was used to compare between treatment groups according to patients' outcomes reporting odds ratios, 95% confidence intervals and p values (objective 4). All tests were of two-sided nature, and a p value less than 0.05 was considered statistically significant. Competing-risk survival regression was performed to adjust for death as a competing event. Statistical analyses were conducted using Stata (version 12) College Station, Texas: StataCorp LP.

Patients who were missing results of VNA, VNP, WBA with AA or WBA with ADP at one or both timepoints were excluded only from the analyses that used these results. For example, if patients were missing the results WBA with AA, they were excluded from diagnostic accuracy analyses and analyses that stratified by WBA with AA but are included in analyses that stratify by VNA and VNP. All the missing test results were either due to technical issue while performing the assay or enrollment error by research staff where an assay was not performed.

The study sample size was determined to detect a presumed change in VNA and VNP after platelet transfusion, as well as literature precedent for standard deviation for these assays.[5,18] For 90% power, alpha 0.05, a sample size of 45 patients postreversal would be required, necessitating 110 patients on antiplatelet therapy anticipating a 41% reversal rate based on a review of our institutional practice over the preceding 2 years.

RESULTS

Patient Population

A total of 223 patients were enrolled and 221 participants were eligible for final analysis (Fig. 1). One hundred eleven were on no antiplatelet medication, 78 were on aspirin, 6 were on clopidogrel and 26 were on both aspirin and clopidogrel. Of patients on aspirin, 5 were taking 325 mg daily, 10 were taking an unknown dose, and the rest were taking 81 mg daily. Demographic characteristics, injury information, pertinent admission laboratories, and transfusion information are shown in Table 1. The median age was 69 years (IQR, 56–78 years) and 120 (54.3%) were women. Patients on aspirin, clopidogrel or both were older. One hundred fifty-eight (71.5%) patients had their initial head CT performed at an outside hospital and presented to our institution as a

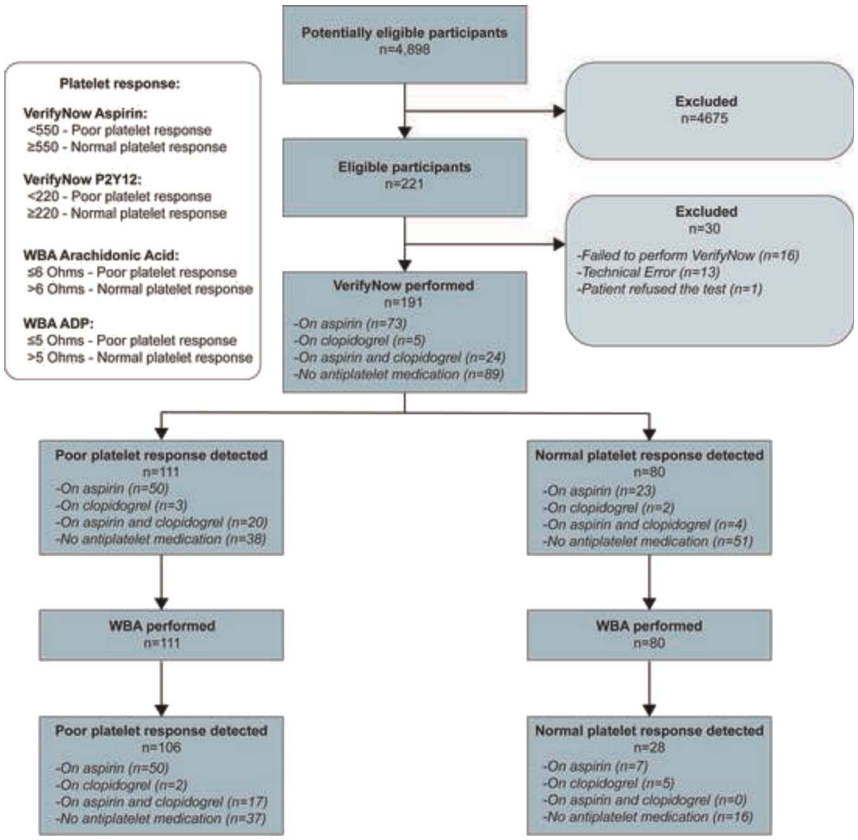

Figure 1. Flow of study participants.

transfer. Glasgow Coma Scale (GCS) score on arrival and Injury Severity Score (ISS) were similar for all patients. Lower hemoglobin levels were seen in patients on clopidogrel (median, 12.1 g/dL; IQR, 11.6–13.2 g/dL) and aspirin and clopidogrel (median, 12.7 g/dL; IQR, 11.2–14.2 g/dL) compared with patients on no antiplatelet medication (median, 13.9 g/dL; IQR, 13.0–15.0 g/dL). Platelet count, international normalized ratio (INR) and activated partial thromboplastin time were similar between all groups. Patients on no antiplatelet medication were less likely to receive a platelet or whole blood transfusion (8.1% for no antiplatelet group vs. 19.2% for aspirin only, 33.3% for clopidogrel only, and 42.3% for the aspirin and clopidogrel groups; $p = 0.025$) and had similar rates of operative intervention. Patients on antiplatelet medication had significantly more cardiovascular comorbidities (Supplemental Table 1, http://links.lww.com/TA/C166). The AIS scores of different body regions were determined and confirmed isolated head injury pattern for all of the study patients (Supplemental Table 2, http://links.lww.com/TA/C167).

Diagnostic Performance of VerifyNow Aspirin and VerifyNow PRUTest

Platelet function testing was successfully performed on 194 (87.8%) participants. For patients on aspirin, VNA revealed that 70 of 97 (72.2%) and 58 of 90 (64.4%) had nonresponsive platelets at 0 and 6 hours, respectively, while WBA with AA as agonist revealed 74/90 (82.2%) and 71/83 (85.5%) to have nonresponsive platelets at 0 hour and 6 hours (Figs. 2A and B). For patients on clopidogrel, VNP revealed that 19 of 28 (67.9%) and 14 of 25 (56.0%) had nonresponsive platelets at 0 hour and 6 hours, respectively, and WBA with ADP as agonist revealed 24 of 27 (88.9%) and 21 of 24 (87.5%) to have nonresponsive platelets at 0 hour and 6 hours (Figs. 2C and D). The sensitivity, specificity, PPV, and NPV of VNA to detect poor platelet response at the time of presentation to the trauma bay, in patients who were taking aspirin, were 77.5%, 58.3%, 91.7%, and 30.4%, respectively. The sensitivity, specificity, PPV, or NPV of VNP for patients taking P2Y12 inhibitors could not be determined because of lack of cases that showed nonresponsive platelets on both VNP and WBA with ADP. The kappa statistic, used to measure the degree of agreement, was 0.259 for VNA and WBA with AA indicating "fair" agreement[19] and −0.24 for VNP and WBA with ADP, indicating significant disagreement between tests.

Poor Platelet Response in Patients Who Were Not Taking Antiplatelet Medication

Among patients on no antiplatelet medication, the prevalence of nonresponsive platelets was 27 of 91 (29.7%) and 22 of 83 (26.5%) based on VNA at 0 hour and 6 hours, 67 of 99 (67.7%) and 55 of 92 (59.8%) based on WBA with AA at 0 hour and 6 hours, 21 of 82 (25.6%) and 17 of 78 (21.8%) based on VNP at 0 hour and 6 hours and 69 of 98 (70.1%) and 50 of 90 (55.6%) based on WBA with ADP at 0 hour and 6 hours (Supplemental Fig. 1, http://links.lww.com/TA/C169).

TABLE 1. Demographics, Injury Information, Laboratory Values and Transfusion Information of the Study Patient Cohort

	All (N = 221)	No Antiplatelet Medication (n = 111), Group 0	Aspirin Only (n = 78), Group 1	Clopidogrel Only (n = 6), Group 2	Aspirin and Clopidogrel (n = 26), Group 3	*p*	Post hoc Differences
Age	69 (56–78)	58 (40–70)	76.5 (68–84)	69.5 (67–72)	74.5 (66–78)	0.0001	0 vs. 1: $p < 0.0001$, 0 vs. 3: 0.0001,
Sex (female)	120 (54.3)	66 (59.5)	40 (51.3)	2 (33.3)	12 (46.2)	0.364	—
Time from injury to trauma bay (min)	278 (121–624)	276 (103–629)	239 (119–539)	238 (233–808)	346 (238–670)	0.385	—
Initial head CT at OSH	158 (71.5)	73 (65.8)	58 (74.4)	6 (100)	21 (80.8)	0.152	—
GCS on arrival	15 (14–15)	15 (14–15)	15 (14–15)	15 (12–15)	15 (13–15)	0.977	—
ISS	10 (9–20)	10 (9–20)	10 (9–17)	10.5 (5–14)	16.5 (9–25)	0.196	—
SAH	103 (65)	48 (65.8)	38 (64.4)	2 (40)	15 (75)	0.533	
SDH	78 (49.7)	42 (57.5)	23 (24)	4 (80)	9 (45)	0.092	
IVH	12 (7.6)	3 (4.1)	8 (13.6)	4 (80)	1 (5)	0.218	
IPH	20 (12.7)	9 (12.3)	10 (16.7)	0 (0)	1 (5)	0.580	
EDH	1 (1.4)	0 (0)	0 (0)	0 (0)	0 (0)	1.00	
Current smoker	47 (22.6)	26 (25.2)	13 (17.3)	1 (16.7)	7 (29.2)	0.499	—
Platelet Count ($\times 10^9$/L)	214 (171–257)	214 (175–253)	216 (171–261)	231 (198–305)	202 (157–242)	0.719	—
INR	1.1 (1–1.1)	1.1 (1–1.1)	1.1 (1–1.1)	1.1 (1.1–1.1)	1.1 (1–1.2)	0.987	—
PTT (sec)	28.2 (26.1–30.7)	28.1 (26–30.4)	28.3 (26.1–31.2)	26.1 (26.1–29.6)	29.1 (26.2–31.8)	0.654	—
Hemoglobin (g/dL)	13.7 (12.5–14.8)	13.9 (13–15)	13.8 (12.4–14.6)	12.1 (11.6–13.2)	12.7 (11.2–14.2)	0.003	0 vs. 2: $p = 0.0515$, 0 vs. 3: 0.005, 1 vs. 2: $p = 0.104$
Creatinine (mg/dL)	0.9 (0.74–1.1)	0.9 (0.7–1)	0.9 (0.74–1.1)	1 (0.95–1.2)	1 (0.75–1.3)	0.623	—
Platelets/WB given (Y)	37 (16.7)	9 (8.1)	15 (19.2)	2 (33.3)	11 (42.3)	0.0001	0 vs. 1: $p = 0.028$, 0 vs. 3: $p < 0.0001$, 1 vs. 3: $p = 0.034$
Type of blood product						0.025	
Platelets	31 (83.8)	5 (55.6)	14 (93.3)	2 (100)	10 (90.9)		
Whole blood	4 (10.8)	4 (44.4)	0 (0)	0 (0)	0 (0)		
Both	2 (5.4)	0 (0)	1 (6.7)	0 (0)	1 (9.1)		
Quantity of blood product						0.548	—
1 Unit	17 (46)	3 (33.3)	8 (53.3)	2 (100)	4 (36.4)		
2 Units	18 (48.6)	5 (55.6)	7 (46.7)	0 (0)	6 (54.5)		
3 Units	2 (5.4)	1 (11.1)	0 (0)	0 (0)	1 (9.1)		
Operative intervention	25 (11.3)	13 (11.7)	6 (7.7)	1 (16.7)	5 (19.2)	0.299	

Data are reported as median (IQR), or percentage.

OSH, outside hospital; SAH, subarachnoid hemorrhage; SDH, subdural hematoma; IVH, intraventricular hemorrhage; IPH, intraparenchymal hemorrhage; EDH, epidural hematoma; PTT, partial thromboplastin time; WB, whole blood.

Impact of Platelet and/or Whole Blood Transfusion on VNA, VNP, and WBA Results

For patients on aspirin who received platelets or whole blood, median (IQR) VNA was 488 (434–551) and 519 (454–568) before and after transfusion, respectively ($p = 0.095$), while WBA with AA was 0.2 (0–0.6) and 0.2 (0.2–1.0) ($p = 0.928$). For patients on clopidogrel who received platelets or whole blood, median (IQR) VNP was 155 (134–250) and 189 (134–251) before and after transfusion, respectively ($p = 0.053$), while WBA with ADP was 0.2 (0.2–1.4) and 0 (0–7) ($p = 0.555$) (Fig. 3). Our results, therefore, indicate a trend toward better platelet response following transfusion, which is not statistically significant.

Impact of VNA-, VNP-, and WBA-Diagnosed Poor Platelet Response on Clinical Outcomes

In our study cohort, 14 (6.3%) patients suffered in-hospital mortality with 4 (1.8%) dying within the first 24 hours. Fifty-six patients (26.2%) had tICH progression, and 58 (27.2%) were discharged with neurologic impairment. A total of 83 (37.6%) of patients were admitted to the ICU with median (IQR) length of ICU stay being 3 days (1–6). Median (IQR) total hospital stay was 3 days (2–7). One hundred twenty-three (55.7%) patients were discharged home, 42 (19.0%) to skilled nursing facility, 39 (17.7%) to TBI rehabilitation program, 2 (0.9%) left against medical advice, and 1 (0.45%) was transferred to inpatient psychiatric ward.

Outcomes were stratified by responsive and nonresponsive platelets and are summarized in Table 2. Importantly, there were no significant differences in any clinical outcome between patients in either group. These results are consistent when comparing responsive versus nonresponsive platelets in patients on no antiplatelet medications (Supplemental Table 3, http://links.lww.com/TA/C168). Among patients receiving aspirin, 24-hour mortality (1/27 [3.7%] vs. 0/68 [0.0%], $p = 0.290$), in-hospital mortality (2/27 [7.4%] vs. 1/68 [1.5%], $p = 0.127$), hemorrhage

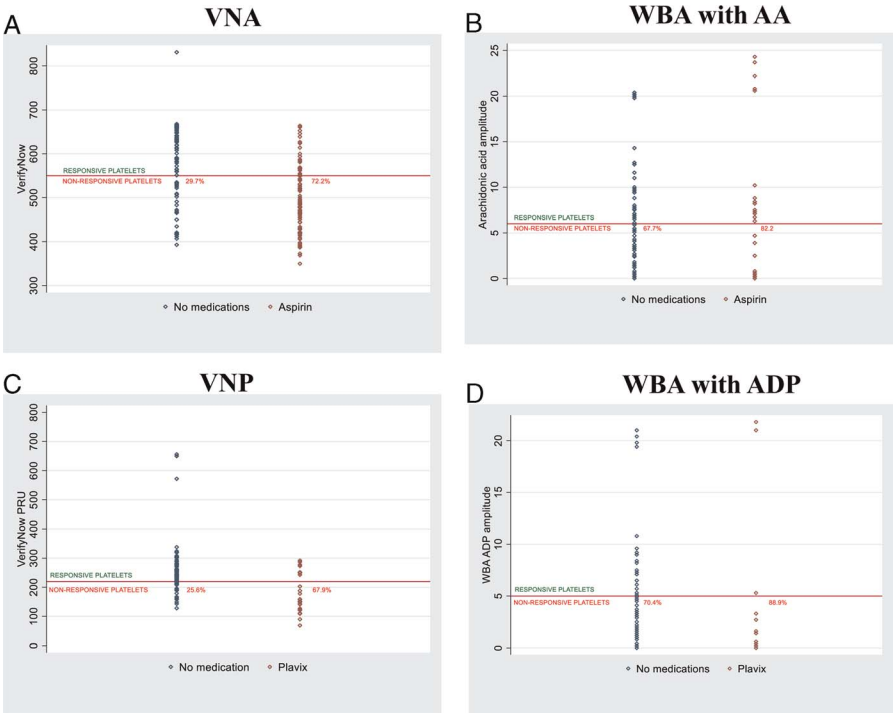

Figure 2. VerifyNow aspirin, VerifyNow P2Y12, whole blood aggregometry with arachidonic acid and whole blood aggregometry with ADP at the time of arrival in the trauma bay.

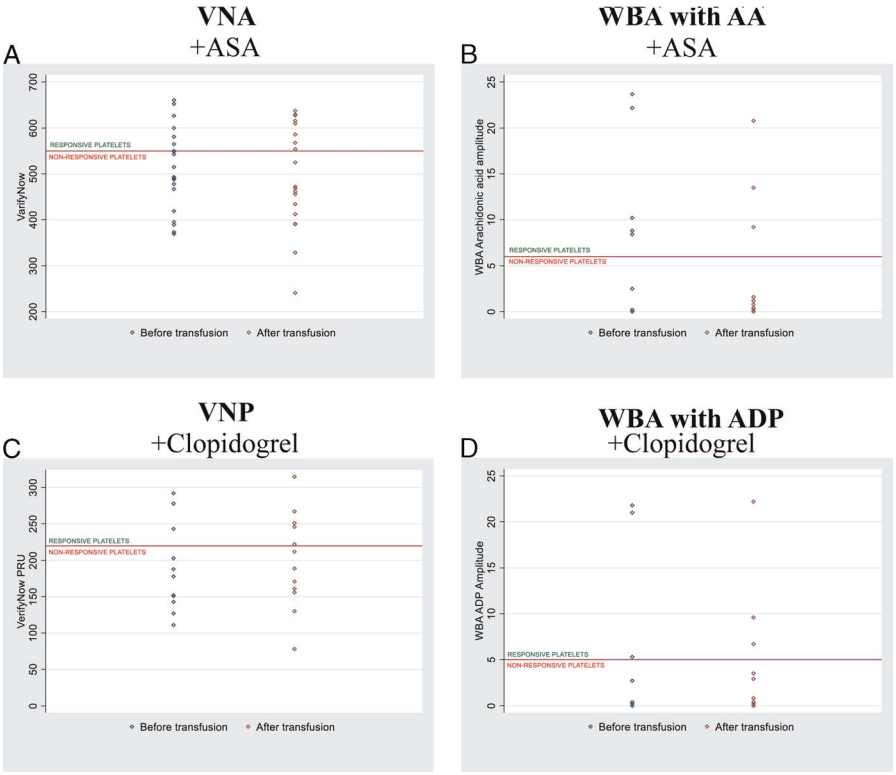

Figure 3. VerifyNow aspirin, VerifyNow P2Y12, whole blood aggregometry with arachidonic acid and whole blood aggregometry with ADP before and after platelet transfusion.

TABLE 2. Clinical Outcomes Stratified by VerifyNow and WBA

	Nonresponsive Platelets on VerifyNow, n = 100	Responsive Platelets on VerifyNow, n = 95	*p*
Mortality at 24 h	1 (1.0)	1 (1.1)	1.00
In-hospital mortality	3 (3.0)	6 (6.3)	0.324
Hemorrhage progression	26 (27.1)	24 (26.1)	0.877
ICU admission	34 (34.3)	38 (40)	0.415
ICU stay for those admitted (d)	2.5 (1–5)	3 (1–7)	0.482
Total hospital stay (d)	3 (2–8)	3.2 (2–7)	0.818
D/c other than home	46 (46.5)	38 (40)	0.387
	Nonresponsive Platelets on WBA, n = 145	**Responsive Platelets on WBA, n = 49**	
Mortality at 24 h	3 (2.1)	0 (0)	0.572
In-hospital mortality	7 (4.9)	3 (6.1)	0.716
Hemorrhage progression	40 (28.4)	8 (17.4)	0.139
ICU admission	52 (36.1)	18 (36.7)	1.00
ICU stay for those admitted (d)	3 (1–6)	3 (1–9)	0.203
Total hospital stay (d)	3 (2–7.3)	3 (2–5.6)	0.638
D/c other than home	65 (45.1)	21 (42.9)	0.868

Data are reported as median (IQR), or percentage.

This table includes patients on aspirin, clopidogrel and patients on no antiplatelet medication. Patients with nonresponsive platelets on either VNA or VNP (or both) are counted as nonresponsive. Patients with responsive platelets on both VNA and VNP are counted as responsive. Similarly, patients with nonresponsive WBA with AA or WBA with ADP result are counted as nonresponsive while patients with responsive platelets on both WBA with AA or WBA with ADP are counted as responsive.

D/c, discharge.

progression (4/27 [14.8%] vs. 16/68 [23.5%], $p = 0.347$), admission to ICU (10/27 [37.0%] vs. 20/68 [29.4%], $p = 0.528$), ICU length of stay (LOS) (2.5 vs. 3 days, $p = 0.971$), total hospital LOS (4 vs. 3 days $p = 0.853$), or discharge other than home (17/27 [63.0%] vs. 32/68 [47.1%], $p = 0.204$) were not different between responsive and nonresponsive platelet groups as measured by VNA. When measured by WBA with AA among the same patients, 24-hour mortality (0/15 [0.0%] vs. 1/74 [1.4%], $p = 1.000$), in-hospital mortality (2/15 [13.3%] vs. 2/74 [2.7%], $p = 0.085$), hemorrhage progression (3/15 [20.0%] vs. 15/74 [20.2%], $p = 0.981$), admission to ICU (7/15 [46.7%] vs. 22/74 [29.7%], $p = 0.149$), ICU LOS (3 vs. 3 days, $p = 0.791$), total hospital LOS (3 vs. 3 days, $p = 0.873$), or discharge other than home (10/15 [66.7%] vs. 38/74 [51.4%], $p = 0.245$) were not different between responsive and nonresponsive platelet groups. Among patients receiving clopidogrel, 24-hour mortality (0/9 vs. 0/18), in-hospital mortality (1/9 [11.1%] vs. 2/18 [11.1%], $p = 0.963$), hemorrhage progression (2/9 [22.2%] vs. 2/18 [11.1%], $p = 0.444$), admission to ICU (3/9 [33.3%] vs. 9/18 [50.0%], $p = 0.673$), total hospital LOS (4 vs. 4 days, $p = 0.700$), or discharge other than home (6/9 [66.7%] vs. 10/18 [55.6%], $p = 0.661$) were not different between responsive and nonresponsive platelet groups as measured by VNP. Intensive care unit LOS was significantly higher in patients with responsive platelets (6 vs. 2 days, $p = 0.027$). When measured by WBA with ADP among patients on clopidogrel, 24-hour mortality (0/3 vs. 0/24), in-hospital mortality (1/3 [33.3%] vs. 2/24 [8.3%], $p = 0.194$), hemorrhage progression (0/3 [0.0%] vs. 3/24 [12.5%], $p = 0.595$), admission to ICU (3/3 [100.0%] vs. 9/24 [37.5%], $p = 0.085$), ICU LOS (2 vs. 3, $p = 1.000$), total hospital LOS (5 vs. 3, $p = 0.534$), discharge other than home (3/3 [100.0%] vs. 12/24 [50.0%], $p = 0.238$) were not different between responsive and nonresponsive platelet groups.

Univariate outcomes of patients who received either platelet transfusion or whole blood transfusion are compared with outcomes of patients who did not (Table 3). Patients who received a transfusion had similar rates of hemorrhage progression (odds ratio [OR], 3.31; 95% confidence interval [CI], 0.99–11.03; $p = 0.052$) and significantly higher rates of admission to ICU (OR, 7.60; 95% CI, 2.02–28.60; $p = 0.003$) compared with patients who did not, after adjusting for age, sex, time between injury and arrival to the trauma bay, GCS score on arrival, AIS head score, preinjury use of aspirin, preinjury use of clopidogrel, platelet count, INR, and creatinine on a multiple logistic regression model. Because of the possibility that some of the patients may have suffered mortality prior to experiencing the measured outcomes, we performed competing-risks regression modeling for ICU admission and prolonged hospital LOS (dichotomized using median as the cutoff). After adjusting for age, ISS, trauma bay GCS, and time between injury and presentation to our hospital, we found that patients with nonresponsive platelets as detected by VerifyNow had similar ICU admission rates (subdistribution hazard ratio [SHR] 1.29, 95% confidence interval [95% CI] 0.77–2.15, $p = 0.333$) and prolonged LOS rates (SHR, 1.33; 95% CI, 0.84–2.11; $p = 0.226$) compared with patients with responsive platelets. When using WBA to measure platelet response, patients with nonresponsive platelets also had similar ICU admission rates (SHR, 1.18; 95% CI, 0.70–1.99; $p = 0.539$), and prolonged LOS rates (SHR, 1.14; 95% CI, 0.71–1.83; $p = 0.60$).

DISCUSSION

We demonstrate that poor platelet response is highly prevalent in patients with isolated tICH, regardless of their antiplatelet medication status. Discrepancy between POC platelet function measurement and laboratory-based aggregometry was evident with only fair correlation between VerifyNow and whole blood aggregometry (Cohen's κ coefficient = 0.259)[19] for patients with tICH on aspirin. Strikingly, a substantial number of patients with isolated TBI taking no antiplatelet agent demonstrated evidence of poor platelet response on laboratory testing, with whole blood aggregometry suggesting as many as 67.7% patients had inadequate response to agonists while the percentage was lower, yet surprisingly prevalent at 29.7% with VerifyNow. However, although significant impairment in platelet response is present on both tests, the clinical significance of these results is unclear as neither were strong predictors of outcome. Detection of poor platelet response was not associated with risk of progression of intracranial hemorrhage.

Platelets are one of the earliest responders to injury and are essential to hemostasis. In patients with intracranial injury, determining platelet function is critical to guide management and holds, in principle, great promise to improve outcomes. However, there is little evidence to support the use of the currently used platelet function tests. In addition, traumatic injury and TBI

TABLE 3. Clinical Outcomes Stratified by Whether Patients Who Were Aspirin or P2Y12 Inhibitor Received Platelet Transfusion

	+ASA +Platelets n = 26	+ASA −Platelets n = 78	*p*	+Clopidogrel +Platelets n = 13	+Clopidogrel −Platelets n = 19	*p*
Mortality at 24 h	1 (3.85)	2 (2.56)	1.00	1 (7.69)	0 (0)	0.406
In-hospital mortality	4 (15.4)	3 (3.9)	0.064	3 (23.1)	1 (5.3)	0.279
Hemorrhage progression	7 (29.2)	16 (20.5)	0.375	3 (25)	2 (10.5)	0.350
ICU admission	20 (76.9)	16 (20.5)	0.0001	13 (100)	2 (10.5)	0.0001
ICU stay, d	3 (2–8)	3 (1.5–3)	0.292	2 (1–3)	5.5 (1–10)	0.862
Total hospital stay, d	7 (3–13)	3 (2–7)	0.007	7 (3–12)	2 (1–5)	0.018
Discharge other than home	20 (76.9)	37 (47.4)	0.009	12 (63.16)	7 (36.84)	0.003

Data are reported as median (IQR), or percentage.

in particular are known to lead to significant platelet function derangements[12] but whether VerifyNow can reliably detect it is unknown. Lastly, VerifyNow and other platelet function tests have been integrated into clinical practice to guide platelet transfusion, but two recent systematic reviews suggest that this practice may not improve outcomes.[20,21] In fact, a large multicenter prospective observational study suggests that taking antithrombotic medication may not worsen outcomes in TBI in a similar patient cohort to the present analysis.[2]

In theory, testing platelet function of patients with tICH upon arrival in the trauma bay is a reasonable strategy to shepherd a valuable resource and improve outcomes. This approach may also be useful to identify patients on aspirin or P2Y12 inhibitors for whom medication history is not available at the time of presentation. However, results of VerifyNow in tICH should be interpreted with caution because of high prevalence of poor platelet response even for patients not on any antiplatelet medication. Presence of apparent antiplatelet effect of VerifyNow does not necessarily identify a patient on antiplatelet medication. The ability of VerifyNow to detect "endogenous" decreased platelet response to agonists after trauma may be a valuable asset of the test; however, the clinical significance remains unclear as discussed. The observation that VerifyNow has only moderate correlation with WBA is not surprising. Several studies have found that VerifyNow does not correlate well with light transmission aggregometry[22,23] or with multiple electrode aggregometry[23] among healthy volunteers or patients with cardiovascular disease. In this study, we demonstrate that VerifyNow has only fair correlation among patients with tICH as well. However, it is equally possible that WBA underestimates platelet response in patients not taking antiplatelet agents, thus it is equally feasible that VerifyNow is a more accurate predictor of poor platelet function. Importantly, neither the observation of poor platelet response on VerifyNow nor WBA demonstrated an association with relevant clinical outcomes, both for patients with antiplatelet medication use as well as for those without. This supports the findings of a recent study of patients with minor injuries, where platelet inhibition as measured by thromboelastography with platelet mapping was frequent with no apparent impact on clinical outcome.[24] Indeed, a recent review highlights that decreased platelet aggregation may be a normal physiologic response, and all ex vivo platelet function testing may be flawed because of the absence of endothelium and flow conditions.[9] These results call into question the clinical significance of a nonresponsive platelet finding on POC testing.

In addition, platelet function testing has been used to guide administration of antiplatelet effect reversal, such as platelet transfusion or desmopressin. Although this is a common practice in the care of trauma patients, most trauma centers have their own highly heterogeneous protocols to direct clinicians. The practices vary because no rigorous prospective study exists to address this urgent clinical question. Studies investigating platelet transfusion in patients on antiplatelet medication presenting with TBI have yielded conflicting results.[25–27] Strikingly, the results of our study suggest that platelet transfusion may not be effective in improving platelet function as measured by VerifyNow or WBA (Fig. 3, Supplemental Figure 1, http://links.lww.com/TA/C169).

Our study has several limitations. First, we chose whole blood aggregometry, which measures change in impedance as the reference test for VerifyNow. Light transmittance aggregometry in platelet-rich plasma is the classic assay for measuring platelet function.[28] Whole blood aggregometry is an updated version that better replicates the in vivo platelet aggregation conditions by using citrated whole blood, is technically simpler, is easier to standardize, and is becoming the new criterion standard platelet function test.[29] It is important to understand, however, that platelet function testing is an emerging field with multiple dissimilar technologies being used with ongoing, but incomplete, efforts toward a single standardized approach. Regarding measurement of tICH progression, our study protocol did not mandate a specific methodology for assessing progression nor was this monitored for adherence, which presents a limitation for assessing this outcome. It is also important to note that while this study was powered to assess the response to platelet transfusion, an a priori power analysis was not done for all of the analyses performed and some of the findings, such as no observed difference in clinical outcomes between patients with responsive and nonresponsive platelets, may be limited by small sample size. Because only five patients on aspirin were taking 325 mg daily, we are unable to determine how a higher dose of aspirin affects platelet function and tICH patient outcomes. It is also notable that a significant proportion of patients in our study were transferred from an outside hospital and therefore may have received interventions after their injury but prior to arrival at our center that may impact the platelet function testing results. In addition, we observed lower than expected rates of platelet transfusion in this cohort, and as such we were underpowered to detect differences in platelet function testing pretransfusion and posttransfusion. Lastly, antiplatelet medications are platelet inhibitors. VerifyNow is designed to detect

platelet inhibition due to antiplatelet medication, which is not part of normal posttrauma physiology. However, our results suggest that VerifyNow or WBA may be detecting the physiologic platelet "exhaustion" or antiplatelet medication-induced "inhibition" and we cannot distinguish between the two with the present assay. Many clinical laboratories, including our own, will use words like "inhibition" and "dysfunction due to presence of aspirin" in the results interpretation, but the current findings suggest that this interpretation may be incomplete. Whether lack of response to an agonist after trauma is adaptive or maladaptive is a topic of debate.[9]

Future research needs to carefully examine several practices that have been adopted for use in trauma without high quality evidence. First, while the present study describes the utility of VerifyNow to guide platelet transfusion, there are additional platelet function tests available in trauma care including thromboelastography with platelet mapping. Similar prospective studies elucidating the ability of thromboelastography with platelet mapping to detect platelet responsiveness and guide transfusion in TBI are needed. Lastly, it is essential to also investigate the impact of platelet transfusion on TBI patient outcomes in a randomized controlled trial, as a recent review of the evidence suggests that this therapy may have limited benefit.[20] In addition, we believe that development of new platelet function tests is urgently needed to improve patient outcomes. These tests should focus on specific aspects of platelet functionality that go beyond platelet aggregation ex vivo, with particular attention to the more recently discovered platelet functions, such as interactions with other blood cells, as well as proinflammatory signaling. Viscoelastic and microfluidic-based platforms may also provide more complete and relevant information.

In conclusion, we find that VerifyNow and WBA detect decreased platelet responsiveness among patients with tICH, irrespective of their use of antiplatelet medication. VerifyNow correlated fairly with whole blood aggregometry among patients with tICH and poor platelet response detectable by these tests did not correlate with clinical outcomes. It is unclear which of these two tests, if either, accurately detect endogenous posttraumatic change in platelet function. In addition, results of this study suggest that platelet transfusion may not improve relevant clinical outcomes in patients with tICH, further raising questions regarding the best practice of platelet function testing.

AUTHORSHIP

J.A. participated in the study design, data collection and analysis, data interpretation, article writing, critical revision. M.Z. participated in the data analysis. I.C. participated in the data collection. J.O.J. participated in the article writing and critical revision. A.H. participated in the data collection. H.P. participated in the data analysis. D.O.O. participated in the study design, critical revision. M.D.N. participated in the study design, data interpretation, article writing, critical revision.

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REFERENCES

1. van den Brand CL, Tolido T, Rambach AH, Hunink MGM, Patka P, Jellema K. Systematic review and meta-analysis: is pre-injury antiplatelet therapy associated with traumatic intracranial hemorrhage? *J Neurotrauma*. 2017;34(1):1–7.
2. Fakhry SM, Morse JL, Garland JM, Wilson NY, Shen Y, Wyse RJ, Watts DD. Antiplatelet and anticoagulant agents have minimal impact on traumatic brain injury incidence, surgery, and mortality in geriatric ground level falls: a multi-institutional analysis of 33,710 patients. *J Trauma Acute Care Surg*. 2021;90(2):215–223.
3. Pamukcu B. A review of aspirin resistance; definition, possible mechanisms, detection with platelet function tests, and its clinical outcomes. *J Thromb Thrombolysis*. 2007;23(3):213–222.
4. Parry PV, Choi PA, Bauer JS, Panczykowski DM, Puccio AM, Okonkwo DO . Utility of the aspirin and P2Y12 response assays to determine the effect of antiplatelet agents on platelet reactivity in traumatic brain injury. *Neurosurgery*. 2017;80(1):92–96.
5. Bachelani AM, Bautz JT, Sperry JL, Corcos A, Zenati M, Billiar TR, Peitzman AB, Marshall GT. Assessment of platelet transfusion for reversal of aspirin after traumatic brain injury. *Surgery*. 2011;150(4):836–843.
6. Gozal YM, Carroll CP, Krueger BM, Khoury J, Andaluz NO. Point-of-care testing in the acute management of traumatic brain injury: identifying the coagulopathic patient. *Surg Neurol Int*. 2017;8:48.
7. Smock KJ, Rodgers GM. Laboratory evaluation of aspirin responsiveness. *Am J Hematol*. 2010;85(5):358–360.
8. Smock KJ, Saunders PJ, Rodgers GM, Johari V. Laboratory evaluation of clopidogrel responsiveness by platelet function and genetic methods. *Am J Hematol*. 2011;86(12):1032–1034.
9. Vulliamy P, Kornblith LZ, Kutcher ME, Cohen MJ, Brohi K, Neal MD. Alterations in platelet behavior after major trauma: adaptive or maladaptive? *Platelets*. 2020;32:295–304.
10. Kornblith LZ, Robles AJ, Conroy AS, Hendrickson CM, Calfee CS, Fields AT, Callcut RA, Cohen MJ. Perhaps it's not the platelet: ristocetin uncovers the potential role of von Willebrand factor in impaired platelet aggregation following traumatic brain injury. *J Trauma Acute Care Surg*. 2019; 85(5):873–880.
11. Nekludov M, Bellander BM, Blombäck M, Wallen HN. Platelet dysfunction in patients with severe traumatic brain injury. *J Neurotrauma*. 2007;24(11): 1699–1706.
12. Kutcher ME, Redick BJ, McCreery RC, Crane IM, Greenberg MD, Cachola LM, Nelson MF, Cohen MJ. Characterization of platelet dysfunction after trauma. *J Trauma Acute Care Surg*. 2012;73(1):13–19.
13. Kim CH, Hwang G, Kwon OK, Ban SP, Chinh ND, Tjahjadi M, Oh CW, Bang JS, Kim T. $P2Y_{12}$ reaction units threshold for implementing modified antiplatelet preparation in coil embolization of unruptured aneurysms: a prospective validation study. *Radiology*. 2017;282(2):542–551.
14. Gengo FM, Rainka M, Robson M, Gengo MF, Forrest A, Hourihane M, Bates V. Prevalence of platelet nonresponsiveness to aspirin in patients treated for secondary stroke prophylaxis and in patients with recurrent ischemic events. *J Clin Pharmacol*. 2008;48(3):335–343.
15. Sternberg Z, Ching M, Sawyer RN, Chichelli T, Li F, Janicke D, Radovic V, Mehta B, Farooq O, Munschauer FE. Clopidogrel responsiveness in stroke patients on a chronic aspirin regimen. *J Stroke Cerebrovasc Dis*. 2013;22(6): 725–732.
16. Neubauer H, Lask S, Engelhardt A, Mügge A. How to optimise clopidogrel therapy? Reducing the low-response incidence by aggregometry-guided therapy modification. *Thromb Haemost*. 2008;99(2):357–362.
17. Ivandic BT, Schlick P, Staritz P, Kurz K, Katus HA, Giannitsis E. Determination of clopidogrel resistance by whole blood platelet aggregometry and inhibitors of the P2Y12 receptor. *Clin Chem*. 2006;52(3):383–388.
18. Kim JT, Heo SH, Choi KH, et al. Clinical implications of changes in individual platelet reactivity to aspirin over time in acute ischemic stroke. *Stroke*. 2015;46(9):2534–2540.
19. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics*. 1977;33(1):159–174.
20. Alvikas J, Myers SP, Wessel CB, Okonkwo DO, Joseph B, Pelaez C, Doberstein C, Guillotte AR, Rosengart MR, Neal MD. A systematic review and meta-analysis of traumatic intracranial hemorrhage in patients taking

prehospital antiplatelet therapy: is there a role for platelet transfusions? *J Trauma Acute Care Surg*. 2020;88(6):847–854.
21. Thorn S, Güting H, Mathes T, Schäfer N, Maegele M. The effect of platelet transfusion in patients with traumatic brain injury and concomitant antiplatelet use: a systematic review and meta-analysis. *Transfusion*. 2019;59(11):3536–3544.
22. Nielsen HL, Kristensen SD, Thygesen SS, Mortensen J, Pedersen SB, Grove EL, Hvas AM. Aspirin response evaluated by the VerifyNow™ Aspirin System and Light Transmission Aggregometry. *Thromb Res*. 2008;123(2):267–273.
23. Gremmel T, Koppensteiner R, Panzer S. Comparison of aggregometry with flow cytometry for the assessment of agonists-induced platelet reactivity in patients on dual antiplatelet therapy. *PLoS One*. 2015;10(6):e0129666.
24. Sirajuddin S, Valdez C, DePalma L, Maluso P, Singhal R, Schroeder M, Sarani B. Inhibition of platelet function is common following even minor injury. *J Trauma Acute Care Surg*. 2016;81(2):328–332.
25. Lokhandwala AM, Asmar S, Khurrum M, Chehab M, Bible L, Castanon L, Ditillo M, Joseph B. Platelet transfusion after traumatic intracranial hemorrhage in patients on antiplatelet agents. *J Surg Res*. 2021;257:239–245.
26. Jehan F, Zeeshan M, Kulvatunyou N, Khan M, O'Keeffe T, Tang A, Gries L, Joseph B. Is there a need for platelet transfusion after traumatic brain injury in patients on P2Y12 inhibitors? *J Surg Res*. 2019;236:224–229.
27. Holzmacher JL, Reynolds C, Patel M, et al. Platelet transfusion does not improve outcomes in patients with brain injury on antiplatelet therapy. *Brain Inj*. 2018;32(3):325–330.
28. Sibbing D, Braun S, Jawansky S, Vogt W, Mehilli J, Schömig A, Kastrati A, von Beckerath N. Assessment of ADP-induced platelet aggregation with light transmission aggregometry and multiple electrode platelet aggregometry before and after clopidogrel treatment. *Thromb Haemost*. 2008;99(1):121–126.
29. Favaloro EJ, Lippi G. *Hemostasis and Thrombosis Methods and Protocols*. 2017.