# Utility of the Aspirin and P2Y12 Response Assays to Determine the Effect of Antiplatelet Agents on Platelet Reactivity in Traumatic Brain Injury

Phillip V. Parry, MD*

Phillip A. Choi, BS‡

Joshua S. Bauer, MS*§

David M. Panczykowski, MD*

Ava M. Puccio, RN, PhD*

David O. Okonkwo, MD, PhD*

*Department of Neurological Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; ‡University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; §St. George's University School of Medicine, Grenada, West Indies

**Correspondence:**
David O. Okonkwo, MD, PhD,
Department of Neurological Surgery,
University of Pittsburgh Medical Center,
200 Lothrop St, Suite B-400,
Pittsburgh, PA 15213.
E-mail: okonkwodo@upmc.edu

**BACKGROUND:** Premorbid antithrombotic medication may worsen intracranial injury and outcome after traumatic brain injury (TBI). Routine laboratory tests are insufficient to evaluate platelet activity.

**OBJECTIVE:** To profile the spectrum of platelet inhibition, as measured by aspirin and P2Y12 response unit assays, in a TBI population on antiplatelet therapy.

**METHODS:** This single-center, prospective cohort study included patients presenting to our institution between November 2010 and January 2015 with a clinical history of TBI. Serum platelet reactivity levels were determined immediately on admission and analyzed using the aspirin and P2Y12 response unit assays; test results were reported as aspirin response units and P2Y12 response units. We report congruence between assay results and clinical history as well as differences in assay results between types of antiplatelet therapy.

**RESULTS:** A sample of 317 patients was available for analysis, of which 87% had experienced mild TBI, 7% moderate, and 6% severe; the mean age was 71.5 years. The mean aspirin response units in patients with a history of any aspirin use was $456 \pm 67$ (range, 350-659), with 88% demonstrating therapeutic platelet inhibition. For clopidogrel, the mean P2Y12 response unit was $191 \pm 70$ (range, 51-351); 77% showed therapeutic response.

**CONCLUSION:** Rapid measurement of antiplatelet function using the aspirin and P2Y12 response assays indicated as many as one fourth of patients on antiplatelet therapy do not have platelet dysfunction. Further research is required to develop guidelines for the use of these assays to guide platelet transfusion in the setting of TBI.

**KEY WORDS:** Aspirin, Aspirin response unit, Clopidogrel, Platelet dysfunction, P2Y12 response unit, Traumatic brain injury
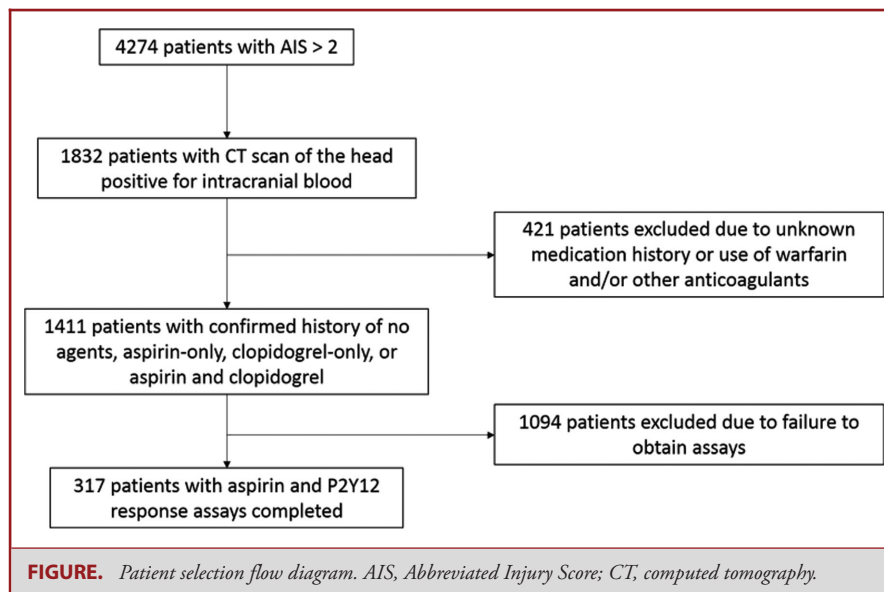
As the population ages, it is more common to encounter patients with traumatic brain injury (TBI) who are on antithrombotic therapy for preexisting illnesses. Premorbid use of antithrombotic medications such as warfarin, aspirin, and clopidogrel may increase the risk and severity of traumatic intracranial hemorrhage and may predispose patients to enlargement of these posttraumatic lesions.[1,2] Standard laboratory analyses are used in patients with TBI to evaluate the degree of anticoagulation due to vitamin K antagonists (ie, warfarin) as well as guide goal-directed reversal of the anticoagulatory effects of these medications to acceptable subtherapeutic levels.[3-7] Unfortunately, these routine coagulation tests are insufficient to evaluate platelet activity and guide hemostatic therapy in TBI patients on antiplatelet therapy.

Recently, point-of-care aspirin response unit (ARU) and P2Y12 response unit (PRU) assays for evaluating aspirin- and thienopyridine-induced platelet inhibition have become widely available and have undergone extensive external validation in the cardiac literature.[8-15] The assays enable platelet function testing for patients taking aspirin, P2Y12 inhibitors (clopidogrel, ticlopidine, or prasugrel), and IIb/IIIa inhibitors (abciximab or eptifibatide)[8-12] and has demonstrated nearly 100% sensitivity and 96% specificity for the detection of antiplatelet

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**ABBREVIATIONS: ARU,** aspirin response unit; **GCS,** glasgow coma scale; **PRU,** P2Y12 response unit; **TBI,** traumatic brain injury

**FIGURE.** *Patient selection flow diagram. AIS, Abbreviated Injury Score; CT, computed tomography.*

function.[16] However, there are currently insufficient data demonstrating the utility of the ARU and PRU assays in assessing platelet function in patients with TBI. In the current study, we evaluated the ARU and PRU assays for the detection of platelet inhibition in TBI patients on premorbid antiplatelet agents.

## METHODS

### Study Population

The study was performed under a Quality Assurance project through the Department of Neurosurgery. Deidentified data were extracted, including patient age, sex, mechanism of injury, Glasgow Coma Scale score, Marshall score, and history of aspirin or clopidogrel use. Patients eligible for this analysis were adults presenting to our institution between November 2010 and January 2015 within 24 hours of a traumatic injury and having a positive clinical screen for TBI necessitating a noncontrast head computed tomography according to American College of Emergency Physicians/Centers for Disease Control and Prevention evidence-based joint practice guidelines.[17] Patients were excluded from analysis if they were younger than 16 years of age, had missing admission computed tomography head scans, had a known bleeding diathesis, were taking anticoagulation therapy (eg, warfarin, dabigatran), and/or in whom antithrombotic agent use was unknown. Patients were also excluded if antiplatelet reversal (eg, platelet transfusion, 1-desamino-8-d-arginine vasopressin administration) was performed at an outside hospital.

### Sample Collection and Measurement of Platelet Function

Platelet dysfunction was evaluated using the VerifyNow assay (Accumetrics, San Diego, CA). During collection of whole-blood samples for standard laboratory tests during presentation to the emergency department, 2 additional tubes of 2 mL of whole blood were collected in standard citrated blood draw collection tubes for the ARU and PRU assays and analyzed using standard, previously described procedures.[9,18] An ARU count <550 was considered therapeutic, whereas a level >550 was considered nontherapeutic and indicated aspirin resistance in patients taking aspirin.[16,19] A PRU count <240 was considered therapeutic, and a level >240 was considered nontherapeutic.[20,21]

### Statistical Analysis

Continuous demographic characteristics were assessed for normality using the Kolmogorov-Smirnov test; normally distributed data were analyzed by a $t$ test, and the remainders were compared using the Wilcoxon rank sum test. Values are reported as mean $\pm$ SD; significant associations between outcomes and predictors are reported as odds ratios with 95% confidence interval. An acceptable type I error was set a priori at $\alpha = .05$ for all statistical tests. All data were analyzed using STATA 13.1 (StataCorp, College Station, Texas).

## RESULTS

### Baseline Characteristics

A total of 317 patients were included in this analysis (Figure). The mean patient age was 71.5 $\pm$ 15.4 years, and 56.5% of patients were male (Table 1). The median Glasgow Coma Scale (GCS) score on admission across all patients was 15 (interquartile range, 14-15). TBI severity on admission was 87% mild (GCS score, 13-15), 7% moderate (GCS score, 9-12), and 6% severe (GCS score ≤8). Intracranial severity as measured by the Marshall score was 2 (interquartile range, 1-2). Mechanisms of injury were 78% fall (either ground level or from a height), 18% motor vehicle accident, and 4% other.

Table 2 reports the distribution of antiplatelet agents in the cohort. The most common antiplatelet therapy was aspirin only (53% of the cohort). Seventeen percent of the cohort was taking a combination of aspirin and clopidogrel, and 5% were taking

**TABLE 1. Baseline Characteristics[a]**

|  | Overall |
|---|---|
| Subjects, N (%) | 317 (100) |
| Age, y, mean ± SD | 71.5 ± 15.4 |
| Sex, male, % | 57 |
| Mechanism of injury, % | |
| Fall (ground level or from height) | 78 |
| Motor vehicle accident | 18 |
| Other | 4 |
| Injury severity | |
| Initial GCS score, median (IQR) | 15 (14-15) |
| Severe TBI (GCS score ≤8), % | 6 |
| Marshall score, median (IQR) | 2 (1-2) |

[a]GCS, Glasgow Coma Scale; IQR, interquartile range; TBI, traumatic brain injury.

**TABLE 2. Distribution of Antiplatelet Agents**

|  | Overall |
|---|---|
| No agent, n (%) | 82 (26) |
| Aspirin only, n (%) | 167 (53) |
| Clopidogrel only, n (%) | 15 (5) |
| Both agents, n (%) | 53 (17) |
| Any aspirin,[a] n (%) | 220 (69) |
| Any clopidogrel,[b] n (%) | 68 (21) |
| Any antiplatelet, n (%) | 235 (74) |
| Total | 317 |

[a]Patients taking either aspirin only or dual therapy.
[b]Patients taking either clopidogrel only or dual therapy.

clopidogrel only. Overall, 69% of the cohort was reported to be taking any aspirin, whereas 21% were taking any clopidogrel. Twenty-six percent of the cohort was not taking any antiplatelet agent at the time of injury.

### ARU and PRU Assay Results

Table 3 demonstrates the results of the ARU and PRU assays compared with the reported clinical history of antiplatelet use. The mean ARU for all patients was 478 ± 82 (range, 350-659), with 79% demonstrating therapeutic platelet inhibition. For clopidogrel, the mean PRU for all patients was 218 ± 74 (range, 51-482), and 62% showed therapeutic response. Eighty-eight percent of patients reporting any aspirin use were therapeutic on ARU, whereas 77% of those on any clopidogrel were therapeutic on PRU; these were significantly greater than those not on antiplatelet therapy ($P < .001$ and $P = .003$, respectively). No significant difference in admission PRU was found between patients who took any aspirin and those taking no agent ($P = .16$). However, a significant difference in ARUs was found between patients who were taking any clopidogrel and those not taking any agent ($P < .001$). Approximately 26% of our patients were not taking an antiplatelet agent, yet 52% and 54% in this cohort had therapeutic assays for aspirin and clopidogrel, respectively.

## DISCUSSION

Many techniques for measuring platelet function have been developed, such as platelet aggregometry, thromboelastography platelet mapping, flow cytometry, Platelet Function Analyzer-100, and urinary 11-dehyrothromboxane $B_2$.[22,23] Platelet aggregometry is considered the historic gold standard. Previous measurements of platelet function were hindered by large-sample volume requirements, sample preparation requirements including centrifuging or pipetting, requirement of specialized personnel, long delays until results, prohibitively expensive equipment, or nonspecific results in the case of measuring thromboxane $B_2$ and its metabolites.[22,23] The ARU and PRU assays can be considered true point-of-care tests, given that they do not have these issues, although there is uncertainty over whether platelet count or hemoglobin level can influence assay results.[11,22,24]

We demonstrated in our cohort of patients that a clinical history of any aspirin or any clopidogrel use is associated with a therapeutic PRU result. However, a substantial proportion of patients with no history of antiplatelet therapy had therapeutic assay results as well (52% with therapeutic ARU and 54% with therapeutic PRU). Our finding of an incongruence between assay results and clinical history is not uncommon. It is well established in the neurosurgical and cardiac literature that a subset of patients with a reported history of antiplatelet therapy will be found to have normal platelet function. In a pair of systematic reviews, Hovens et al[25] and Snoep et al[26] report that approximately 1 in 4 patients taking aspirin will show biochemical resistance, and 1 in 5 patients taking clopidogrel will be resistant. Conversely, platelet dysfunction in patients without a history of antiplatelet use in the setting of TBI is not uncommon.[27,28] Genetic polymorphisms of platelet and cytochrome P-450 enzymes, chronic illness, brain trauma–specific changes in the inflammatory milieu, consumption of platelet granule contents, antiplatelet medications, and other factors likely influence platelet function at the time of injury.[29-32] Our results indicate that the majority of patients had therapeutic ARU and PRU results regardless of a history of platelet use. Both our study and that of Bachelani et al[27] used cutoff thresholds for therapeutic platelets based on the cardiac literature (550 for ARUs and 240 for PRUs), which may not be valid in the TBI population. Future investigation of meaningful thresholds for the ARU and PRU assays in the TBI population using outcomes may be warranted. Previous studies investigating the relationship between a history of antiplatelet therapy and outcome in TBI have had conflicting results, which may be explained by a multitude of factors affecting platelet function.[33-37]

**TABLE 3.** Admission Aspirin Response Unit and P2Y12 Response Unit Results Stratified by Antiplatelet Therapy[a]

| | % Therapeutic, ARU | ARU, Mean (SD) | P, ARU | % Therapeutic, PRU | PRU, Mean SD, | P, PRU |
|---|---|---|---|---|---|---|
| No agent | 52 | 536 (90) | | 54 | 229 (76) | |
| Aspirin only | 87 | 460 (68) | <.001[b] | 53 | 237 (65) | .54 |
| Clopidogrel only | 63 | 518 (97) | .59 | 73 | 210 (64) | .39 |
| Both agents | 92 | 439 (61) | <.001[b] | 78 | 185 (72) | .002[b] |
| Any aspirin (aspirin only + both) | 88 | 456 (67) | <.001[b] | 65 | 212 (73) | .15 |
| Any clopidogrel (clopidogrel only + both) | 88 | 451 (72) | <.001[b] | 77 | 191 (70) | .003[b] |
| Any antiplatelet agent | 87 | 457 (69) | <.001[b] | 66 | 212 (72) | .14 |
| All patients | 79 | 478 (82) | | 62 | 218 (74) | |

[a]ARU, aspirin response unit; PRU, P2Y12 response unit.
[b]Indicates a significant result.
Aspirin-only, clopidogrel-only, and both agent comparisons were made against no agent. For any aspirin, any clopidogrel, and any antiplatelet agent, comparisons were made against patients not taking the particular agent. The Student t test used for all comparisons of assay values.

The ARU and PRU assays have been validated in the cardiac literature to reveal platelet dysfunction and predict future adverse events.[8,9,12-15,38] We show in this study its feasibility in the TBI population. Further work to validate the utility of the ARU and PRU assays in the TBI population as a prognostic and management tool is required.

## Disclosures

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