ORIGINAL RESEARCH

# Clinical Significance of Acute and Serial Platelet Function Testing in Acute Ischemic Stroke

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***Background*—**We sought to investigate the clinical implications of platelet reactivity to aspirin and the variability in the platelet reactivity to aspirin during acute periods for the risk of vascular events in patients with acute ischemic stroke.

***Methods and Results*—**This was a single-center, prospective, observational study. The aspirin reaction unit was blindly measured at the following two times: after 3 hours of aspirin loading and on the fifth day of aspirin administration. High on-aspirin platelet reactivity (HAPR) was defined as an aspirin reaction unit ≥550 IU. The primary outcome measure was the 1-year composite of stroke, myocardial infarction, and vascular death. A total of 805 patients (aged 66±12 years, 61% male) were analyzed in this study. Ninety-nine of 805 (12.3%) patients and 78 of 558 (14.0%) patients had HAPR at the time of the fifth day of aspirin administration and after 3 hours of aspirin loading measurements, respectively. Patients with HAPR than normal on-aspirin platelet reactivity at the fifth day of aspirin administration measurement were more likely to have experienced 1-year vascular event. HAPR at the fifth day of aspirin administration measurement was independently associated with a greater risk of experiencing 1-year vascular event (hazard ratio, 1.84; 95% confidence interval, 1.07–3.19). Moreover, persistently HAPR substantially increased the risk of 1-year vascular events (hazard ratio, 3.11; 95% confidence interval, 1.23–7.86).

***Conclusions*—**These results suggest that HAPR during the acute stage of ischemic stroke increases the risk of subsequent vascular events and that serial aspirin reaction unit measurements may identify patients with acute ischemic stroke who are at a higher risk for vascular events. Additional studies are warranted to determine the appropriate treatments for patients with acute ischemic stroke with HAPR. **(*J Am Heart Assoc*. 2018;7:e008313. DOI: 10.1161/JAHA.117.008313.)**

**Key Words:** aspirin • ischemic • resistance • stroke

Aspirin reduces the risks of recurrent ischemic stroke, major coronary events, and serious vascular events by ≈22%, 20%, and 19%, respectively.[1] Given that the risk of recurrent stroke is highest during the earlier periods after ischemic stroke or transient ischemic attack (TIA),[2] immediate treatment with aspirin is important for preventing vascular events.[3,4]

High on-aspirin platelet reactivity (HAPR), which is also known as biological aspirin resistance, may increase the risks of vascular events and recurrent stroke in ischemic stroke.[5,6] Noncompliance, obesity, and high platelet turnover may cause suboptimal platelet inhibition by aspirin.[7–9] However, only limited information regarding this issue has been collected by previous studies, which assessed platelet reactivity to aspirin before new ischemic stroke or platelet responsiveness to chronic aspirin treatment. Recent studies found that platelet reactivity to antiplatelet medications can change over time in significant proportions of patients.[10,11] Single-platelet function measurements may not consistently reflect platelet responsiveness to aspirin in the acute periods after ischemic stroke.

Therefore, we sought to investigate whether HAPR and changes in aspirin platelet reactivity after consecutive aspirin treatments during the acute period after acute ischemic stroke were associated with the occurrence of subsequent vascular events.

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Accompanying Tables S1, S2 and Figure S1 are available at http://jaha.ahajournals.org/content/7/11/e008313/DC1/embed/inline-supplementary-material-1.pdf

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## Clinical Perspective

### What Is New?

- This prospective study of over 800 ischemic stroke patients treated with aspirin thoroughly explored the clinical implications of acute platelet reactivity related to aspirin using both single and serial aspirin reaction unit measurements.
- High on-aspirin platelet reactivity at the time of the fifth day of aspirin administration measurement was independently associated with an 84% increased risk of the composite of stroke, myocardial infarction, and vascular death.
- In the 558 patients who underwent both 3 hours of aspirin loading and fifth day of aspirin administration measurements, patients with persistent high on-aspirin platelet reactivity were more likely to experience the composite of stroke, myocardial infarction, and vascular events than patients with persistent normal on-aspirin platelet reactivity.

### What Are the Clinical Implications?

- High on-aspirin platelet reactivity during the acute stage of ischemic stroke could increase the risk of subsequent vascular events, and serial aspirin reaction unit measurements may identify patients with acute ischemic stroke who are at a higher risk for vascular events.
- Aspirin resistance after acute aspirin treatment may be a target of alternative antiplatelet therapies and the focus of future clinical research on stroke prevention.

## Methods

The data, analytical methods, and study materials will not be made available to other researchers for the purpose of reproducing the results because of legal regulations regarding access to patient-level data. This was a single-center, prospective, observational study whose patients were consecutively recruited from the Stroke Center of Chonnam National University Hospital between April 2012 and December 2013. The general methods for this study were described previously in 2 previous studies[11,12]; this study used a more-expanded cohort. Patients who met the following criteria were included in the main analysis: (1) patients who presented to our hospital and were evaluated within 3 days of symptom onset; (2) patients who exhibited positive ischemic lesions with an apparent diffusion coefficient; (3) patients who were not at high risk for cardioembolism; and (4) patients who provided written informed consent to participate in the study. The following patients were excluded from this study: (1) patients with other etiologies by the TOAST (Trial of Org 10 172 in Acute Stroke Treatment) classification; (2) patients with malignant infarction; (3) patients lost during follow-up; (4) patients who were chronic nonsteroidal anti-inflammatory drug users (>3 days per week for the past 3 months); (5) patients with a history of hemorrhagic disorder within the past 4 weeks; (6) patients with coagulopathy; (7) patients with thrombocytopenia (<90 000 platelets/μL); (8) patients with a low hematocrit (<29%); and (9) patients with chronic liver or renal disease. The study was approved by the Institutional Review Board of Chonnam National University Hospital, and all of its clinical investigations were conducted in accord with the principles outlined in the Declaration of Helsinki. Written informed consent was obtained from the participants or their families.

## Management

Aspirin was administered to the study patients immediately after brain magnetic resonance imaging. The use of enteric-coated aspirin or intravenous antithrombotics, such as glycoprotein IIb/IIIa inhibitors, was not permitted during the study admission period. The initial loading dose was 300 mg. This dose was followed by daily 100-mg maintenance doses. We ensured that patients received their medication daily during admission period. During the follow-up period, dedicated nurses or physicians conducted face-to-face or telephone interviews every 3 months to investigate medication adherence. A combination of aspirin and other antiplatelet agents was administered to patients with symptomatic arterial steno-occlusion, a previous history of coronary artery disease, or a previous history of antiplatelet treatment at the discretion of the treating physicians. Patients whose atrial fibrillation (or high potential cardioembolic sources) was detected after discharge were prescribed anticoagulants instead of aspirin and were censored in the study.

## Platelet Function Study

The aspirin reaction unit (ARU) was measured using VerifyNow (Accumetrics, San Diego, CA). The ARU was measured 2 times during admission. The acute ARU (aARU) was measured 3 hours after aspirin loading at a dose of 300 mg in the emergency department in patients eligible for the aARU study, and the ARU-5 was measured on the fifth consecutive day of aspirin administration in all patients.[11,12] Both the physicians and patients were blinded to the ARU measurement results. Aspirin resistance, or HAPR, was diagnosed in patients with an ARU value ≥550 IU, which is a commonly used cut-off value. For the post-hoc analysis, patients were divided into the following 4 groups according to whether their HAPR status changed between the aARU and ARU-5 measurements: (1) group 1, which comprised patients who were aspirin responders (normal on-aspirin platelet reactivity [NAPR]; ARU value <550 IU), as determined by the aARU and ARU-5 measurements; (2) group 2, which comprised patients who were aspirin nonresponders (HAPR; aARU value ≥550 IU) at

the time of the aARU measurement but were aspirin responders (ARU-5 value <550 IU) at the time of the ARU-5 measurement; (3) group 3, which comprised patients who were aspirin responders at the time of the aARU measurement but were aspirin nonresponders at the time of the ARU-5 measurement; and (4) group 4, which comprised patients who aspirin nonresponders at the times of the aARU and ARU-5 measurements.

## Outcomes

The primary outcome was a composite of vascular events, including stroke (either ischemic or hemorrhagic), myocardial infarction (MI), and vascular death, up to 1 year after stroke. The secondary outcome was stroke (either ischemic or hemorrhagic) recurrence within 1 year after stroke. Outcomes were blindly assessed by dedicated nurses and either conducted face-to-face or through telephone interviews every 3 months and at 1 year.

## Statistical Analysis

The data are presented as the means and SD (or median and interquartile ranges) for continuous variables or the frequency of categorical variables. Categorical variables were analyzed using the $\chi^2$ test and Fisher's exact test, where appropriate. Continuous variables were analyzed using the independent-sample *t* test or the Mann–Whitney *U* test, where appropriate.

Baseline characteristics were compared between patients with HAPR and patients with NAPR at the time of the ARU-5 measurement. Two additional subgroups were compared to determine whether the aARU measurements predict the occurrence of vascular events and whether the persistence of aspirin resistance at aARU and ARU-5 measurements affects outcomes for the post-hoc analysis. Rates of the primary and secondary outcomes were estimated using the Kaplan–Meier product-limit method and were also compared between patients with HAPR and NAPR at the times of the ARU-5 and aARU measurements among the 4 groups according to the aspirin responder status by the log-rank test. Cox proportional hazard regression analysis was used to evaluate the independent effects of HAPR on outcome event rates. Proportional assumptions were checked by the Supremum test for proportional hazards assumption using cumulative sums of Martingale-based residuals. Adjustments were performed for the following variables according to their clinical significance and the results of previous studies: age, male sex, baseline National Institutes of Health Stroke Score (NIHSS) scores, hypertension, diabetes mellitus, smoking, dyslipidemia, a previous history of stroke, a previous history of aspirin use, platelet counts, blood glucose levels, and the TOAST classifications. To explore whether previous aspirin

**Table 1.** General Characteristics of the Subjects

| | ARU-5<550 IU | ARU-5≥550 IU | *P* Value |
|---|---|---|---|
| N | 706 | 99 | |
| Age, y (mean±SD) | 66±12 | 67±12 | 0.29 |
| Male | 435 (61.6) | 56 (56.6) | 0.42 |
| Onset to visit | | | 0.51 |
| Within 12 h | 428 (60.6) | 64 (64.6) | |
| 12 h to 3 d | 278 (39.4) | 35 (35.4) | |
| Initial NIHSS score (med, IQR) | 2 (1, 5) | 2 (1, 5) | 0.66 |
| Body mass index (mean±SD) | 23.5±3.1 | 23.4±3.4 | 0.74 |
| Hypertension | 397 (56.2) | 52 (52.5) | 0.52 |
| DM | 205 (29.0) | 26 (26.3) | 0.64 |
| Dyslipidemia | 126 (17.8) | 10 (10.1) | 0.06 |
| Smoking | 237 (33.6) | 36 (36.4) | 0.57 |
| Coronary artery disease | 48 (6.8) | 6 (6.1) | >0.99 |
| Previous stroke or TIA | 97 (13.7) | 15 (15.2) | 0.76 |
| Previous antiplatelet medication | | | |
| Aspirin | 161 (22.8) | 19 (19.2) | 0.52 |
| Nonaspirin | 104 (14.7) | 15 (15.2) | 0.88 |
| Laboratory findings (mean±SD) | | | |
| Platelet count | 226±63 | 200±54 | <0.001 |
| LDL-cholesterol | 123±41 | 111±38 | 0.007 |
| Glucose | 141±58 | 140±64 | 0.90 |
| HbA1C | 6.3±1.4 | 6.3±1.4 | 0.59 |
| >6.5 | 201/701 (28.7) | 26/99 (26.3) | 0.72 |
| Reperfusion therapy | 129 (18.3) | 14 (14.1) | 0.40 |
| TOAST | | | 0.15 |
| LAA | 362 (51.3) | 41 (41.4) | |
| SVO | 151 (21.4) | 28 (28.3) | |
| UD | 193 (27.3) | 30 (30.3) | |
| Acute ARU (n=558) | 465±62 | 497±72 | <0.001 |
| ≥550 IU | 57/482 (11.8) | 21/76 (27.6) | 0.001 |
| Medications at discharge | | | |
| Antihypertensives | 346 (49.0) | 54 (54.5) | 0.34 |
| Antidiabetics | 668 (94.6) | 89 (89.9) | 0.07 |
| Statin | 194 (27.5) | 25 (25.3) | 0.72 |
| Combined antiplatelet therapy | 173 (24.5) | 13 (13.1) | 0.01 |

ARU indicates aspirin reaction unit; ARU-5, aspirin reaction unit measured on the fifth consecutive day of aspirin administration; DM, diabetes mellitus; HbA1C, glycated hemoglobin; IQR, interquartile range; LAA, large artery atherosclerosis; LDL, low-density lipoprotein; NIHSS, National Institutes of Health Stroke Score; SVO, small vessel occlusion; TIA, transient ischemic attack; TOAST, Trial of Org 10 172 in Acute Stroke Treatment; UD, undetermined etiology.

**Table 2.** General Characteristics of Patients According to Groups of the ARU Changes

| | aARU<550 IU | aARU≥550 IU | *P* Value | Group 1<br>aARU<550 IU<br>ARU-5<550 IU | Group 2<br>aARU≥550 IU<br>ARU-5<550 IU | Group 3<br>aARU<550 IU<br>ARU-5≥550 IU | Group 4<br>aARU≥550 IU<br>ARU-5≥550 IU | *P* Value |
|---|---|---|---|---|---|---|---|---|
| N | 480 | 78 | | 425 | 57 | 55 | 21 | |
| Age, y | 66±12 | 65±12 | 0.60 | 66.3±11.7 | 63.0±11.9 | 64.1±12.2 | 71.4±11.8 | 0.02 |
| Male | 285 (59.4) | 48 (61.5) | 0.80 | 249 (58.6) | 38 (66.7) | 36 (65.5) | 10 (47.6) | 0.33 |
| Onset to visit | | | 0.39 | | | | | 0.55 |
| Within 12 h | 272 (56.7) | 40 (51.3) | | 241 (56.7) | 27 (47.4) | 31 (56.4) | 13 (61.9) | |
| 12 h to 3 d | 208 (43.3) | 38 (48.7) | | 184 (43.3) | 30 (52.6) | 24 (43.6) | 8 (38.1) | |
| Initial NIHSS (med, IQR) | 2 (1, 3) | 2 (1, 4) | 0.14 | 2 (1, 3) | 3 (1, 5) | 2 (1, 4) | 2 (1, 3) | 0.27 |
| Body mass index (mean±SD) | 23.6±3.1 | 23.9±3.2 | 0.51 | 23.6±3.1 | 24.2±2.9 | 24.0±2.9 | 23.0±3.7 | 0.30 |
| Hypertension | 286 (59.6) | 43 (55.1) | 0.46 | 257 (60.5) | 32 (56.1) | 29 (52.7) | 11 (52.4) | 0.61 |
| DM | 152 (31.7) | 21 (26.9) | 0.43 | 137 (32.2) | 16 (28.1) | 15 (27.3) | 5 (23.8) | 0.71 |
| Dyslipidemia | 76 (15.8) | 9 (11.5) | 0.40 | 70 (16.5) | 9 (15.8) | 6 (10.9) | 0 | 0.17 |
| Smoking | 146 (30.4) | 26 (33.3) | 0.60 | 123 (28.9) | 22 (38.6) | 23 (41.8) | 4 (19.0) | 0.08 |
| Coronary artery diseases | 26 (5.4) | 7 (9.0) | 0.20 | 24 (5.6) | 6 (10.5) | 2 (3.6) | 1 (4.8) | 0.42 |
| Previous stroke or TIA | 71 (14.8) | 14 (17.9) | 0.50 | 61 (14.4) | 10 (17.5) | 10 (18.2) | 4 (19.0) | 0.78 |
| Previous antiplatelet* | | | | | | | | |
| Aspirin | 110 (22.9) | 22 (28.2) | 0.32 | 100 (23.5) | 18 (31.6) | 10 (18.2) | 4 (19.0) | 0.37 |
| Nonaspirin | 74 (15.4) | 15 (19.2) | 0.41 | 66 (15.5) | 10 (17.5) | 8 (14.5) | 5 (23.8) | 0.75 |
| Laboratory findings | | | | | | | | |
| Platelet count | 226±62 | 205±51 | 0.007 | 228±63 | 218±47 | 205±47 | 170±43 | <0.001 |
| LDL-cholesterol | 120±41 | 118±41 | 0.62 | 120±42 | 124±42 | 118±38 | 102±31 | 0.18 |
| Glucose | 145±62 | 134±45 | 0.11 | 146±62 | 132±40 | 143±65 | 138±59 | 0.43 |
| HbA1C (n=555) | | | | | | | | |
| >6.5 | 147 (30.8) | 18 (23.1) | 0.18 | 130 (30.8) | 13 (22.8) | 17 (30.9) | 5 (23.8) | 0.59 |
| TOAST | | | 0.65 | | | | | 0.64 |
| LAA | 248 (51.7) | 36 (46.2) | | 225 (52.9) | 28 (49.1) | 23 (41.8) | 8 (38.1) | |
| SVO | 115 (24.0) | 20 (25.6) | | 98 (23.1) | 14 (24.6) | 17 (30.9) | 6 (28.6) | |
| UD | 117 (24.4) | 22 (28.2) | | 102 (24.0) | 15 (26.3) | 15 (27.3) | 7 (33.3) | |
| Medications at discharge | | | | | | | | |
| Antihypertensives | 243 (50.6) | 41 (52.6) | 0.81 | 216 (50.8) | 29 (50.9) | 27 (49.1) | 12 (57.1) | 0.94 |
| Antidiabetics | 149 (31.0) | 17 (21.8) | 0.11 | 133 (31.3) | 11 (19.3) | 16 (29.1) | 6 (28.6) | 0.32 |
| Statin | 457 (95.2) | 73 (93.6) | 0.57 | 405 (95.3) | 55 (96.5) | 52 (94.5) | 18 (85.7) | 0.24 |
| Combined antiplatelet | 92 (19.2) | 19 (24.4) | 0.29 | 85 (20.0) | 13 (22.8) | 7 (12.7) | 6 (28.6) | 0.38 |

aARU indicates aspirin reaction unit measured 3 hours after aspirin loading; ARU, aspirin reaction unit; ARU-5, aspirin reaction unit measured on the fifth consecutive day of aspirin administration; DM, diabetes mellitus; HbA1C, glycated hemoglobin; IQR, interquartile range; LAA, large artery atherosclerosis; LDL, low-density lipoprotein; NIHSS, National Institutes of Health Stroke Score; SVO, small vessel occlusion; TIA, transient ischemic attack; TOAST, Trial of Org 10 172 in Acute Stroke Treatment; UD, undetermined etiology.

use modified the effects, we generated an interaction term for the relationship between the HAPR at the times of the ARU-5 measurements and previous aspirin and examined its statistical significance using the Cox proportional hazard models. Hazard ratios (HRs) and the corresponding 95% confidence intervals (CIs) were estimated. All *P* values were 2-sided, and statistical significance was defined as a *P* value of less than 0.05. SAS (version 9.4; SAS Institute, Cary, NC) was used for all the statistical analysis.

## Results

A total of 1415 patients with stroke were prospectively screened during the study period. Of these patients, 968 were

**Table 3.** Crude Events in All Cohorts and Subgroups

| | Primary Outcome | *P* Value* | Stroke | *P* Value* | MI | *P* Value* | Vascular Death | *P* Value* |
|---|---|---|---|---|---|---|---|---|
| ARU-5 (n=805) | | 0.048 | | 0.38 | | 0.001 | | 0.37 |
| <550 IU | 73 (10.9) | | 41 (6.0) | | 4 (0.6) | | 35 (5.6) | |
| ≥550 IU | 17 (18.8) | | 8 (8.8) | | 4 (4.7) | | 7 (7.8) | |
| aARU (n=558) | | 0.22 | | 0.42 | | 0.96 | | 0.62 |
| <550 IU | 51 (11.2) | | 31 (6.7) | | 6 (1.4) | | 19 (4.3) | |
| ≥550 IU | 12 (15.8) | | 7 (9.4) | | 1 (1.3) | | 4 (5.6) | |
| ARU changes (n=558) | | 0.095 | | 0.59 | | <0.001 | | 0.028 |
| Both aARU and ARU-5<550 IU | 44 (10.9) | | 27 (6.6) | | 2 (0.5) | | 18 (4.7) | |
| aARU≥550 IU, ARU-5<550 IU | 6 (10.9) | | 4 (7.4) | | 1 (1.9) | | 1 (1.9) | |
| aARU<550 IU, ARU-5≥550 IU | 7 (13.4) | | 4 (8.2) | | 3 (8.1) | | 0 | |
| Both aARU and ARU-5≥550 IU | 6 (28.6) | | 3 (14.8) | | 0 | | 3 (16.7) | |

Primary outcome: composite of stroke, MI, and vascular death. aARU indicates aspirin reaction unit measured 3 hours aspirin loading; ARU indicates aspirin reaction unit; ARU-5, aspirin reaction unit measured on the fifth consecutive day of aspirin administration; MI, myocardial infarction.
*Log-rank test.

candidates for the current study, because they had positive ischemic lesions on the apparent diffusion coefficient and had suffered noncardioembolic strokes within 3 days of onset. Informed consent for participation in the study was provided by 935 patients. Selection of the study population is presented in Figure S1. A total of 805 patients (mean age of 66±12 years; 61% male) were ultimately analyzed. The mean ARU-5 value was 448.8±65.3 IU (median, 424; interquartile range, 354–614). Of the abovementioned 805 patients, 99 (12.3%) had HAPR (ARU-5 value ≥550 IU) at the time of the ARU-5 measurement. With respect to the initial stroke severity, the median NIHSS score was 2 (interquartile

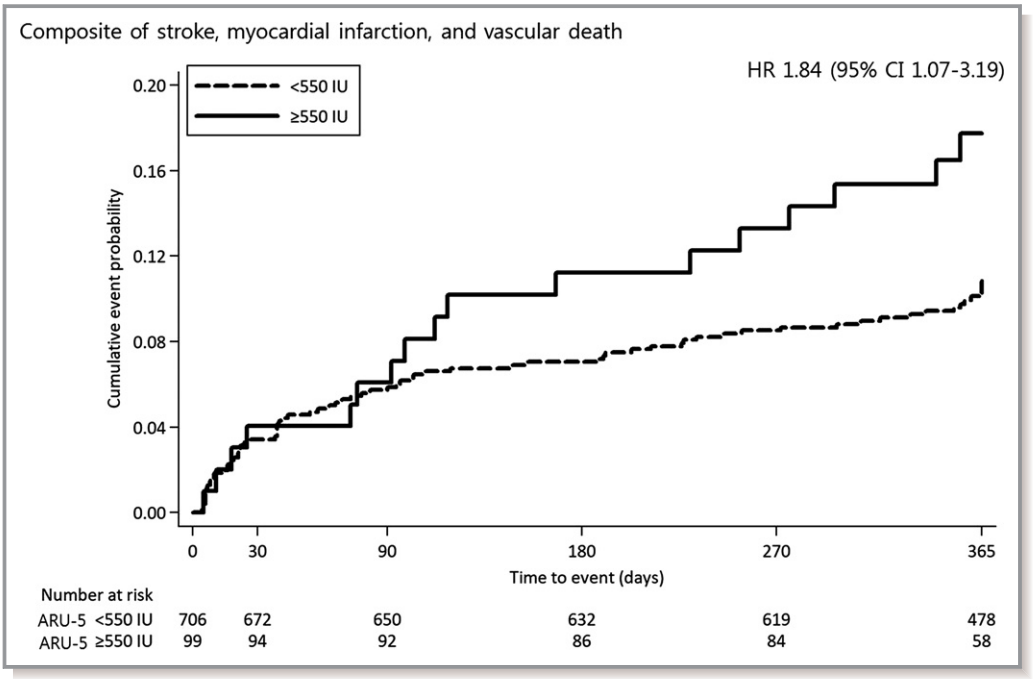


**Figure 1.** Kaplan–Meier curves showing the cumulative probability of the composite of stroke, myocardial infarction, and vascular death according to whether patients had HAPR or NAPR at the time of the ARU-5 measurement. aARU indicates acute aspirin loading; ARU-5, aspirin reaction unit measured on the fifth consecutive day of aspirin administration; CI, confidence interval; HAPR, high on-aspirin platelet reactivity; HR, hazard ratio; NAPR, normal on-aspirin platelet reactivity.

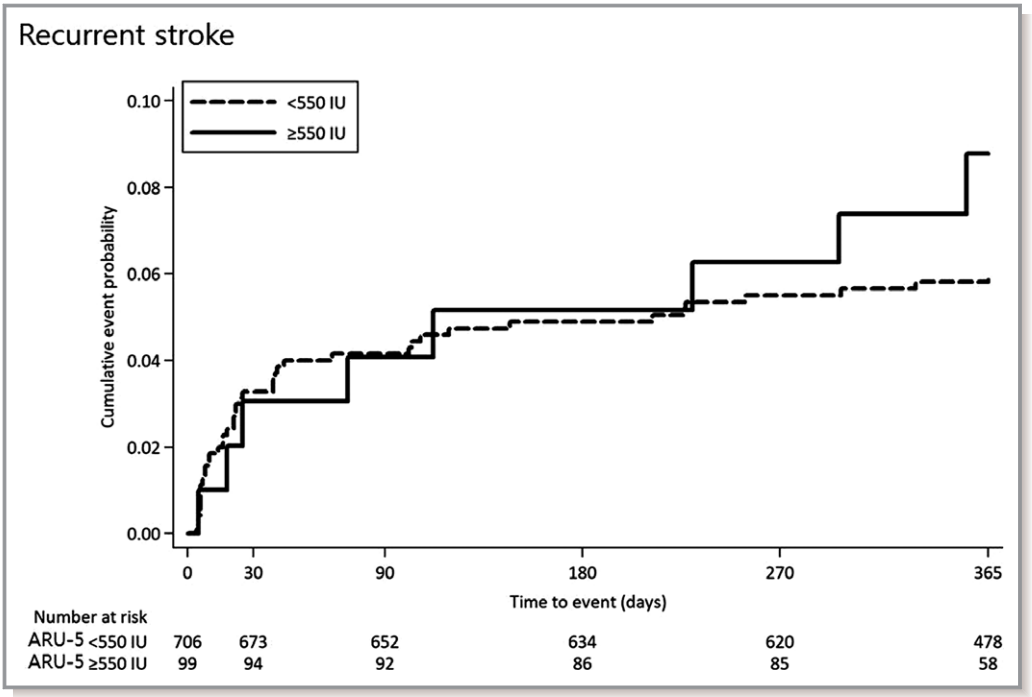


**Figure 2.** Kaplan–Meier curves to show cumulative probability of recurrent stroke according to whether patients had HAPR or NAPR at the time of the ARU-5 measurement. ARU-5 indicates aspirin reaction unit measured on the fifth consecutive day of aspirin administration; HAPR, high on-aspirin platelet reactivity; NAPR, normal on-aspirin platelet reactivity.

range, 0–5). Of the abovementioned 805 patients, 558 (69.3%) underwent an aARU measurement 3 hours after aspirin loading at a dose of 300 mg. Among those patients, 78 (14.0%) had HAPR. Group 1 (aARU and ARU-5 value <550 IU) comprised 425 (76.2%) patients, group 2 (aARU value ≥550 IU and ARU-5 value ≥550 IU) comprised 57 (10.2%) patients, group 3 (aARU value <550 IU and ARU-5 value ≥550 IU) comprised 55 (9.9%) patients, and group 4 (aARU and ARU-5 values ≥550 IU) comprised 21 (3.8%) patients.

**Table 4.** Cox proportional Hazard Regression Analysis for Primary Outcome and Recurrent Stroke

| | Primary Outcome | | | | Stroke Recurrence | | | |
|---|---|---|---|---|---|---|---|---|
| | Crude HR | *P* Value | Adjusted HR | *P* Value | Crude HR | *P* Value | Adjusted HR | *P* Value |
| ARU-5 (n=805) | | 0.05 | | 0.03 | | 0.38 | | 0.33 |
| <550 IU | Ref | | Ref | | Ref | | Ref | |
| ≥550 IU | 1.69 (0.99–2.87) | | 1.84 (1.07–3.19) | | 1.40 (0.66–2.99) | | 1.48 (0.67–3.23) | |
| aARU (n=558) | | 0.23 | | 0.19 | | 0.42 | | 0.34 |
| <550 IU | Ref | | Ref | | Ref | | Ref | |
| ≥550 IU | 1.47 (0.79–2.76) | | 1.55 (0.81–2.98) | | 1.40 (0.62–3.18) | | 1.51 (0.65–3.54) | |
| ARU changes (n=558) | | | | | | | | 0.57 |
| Both aARU and ARU-5<550 IU | Ref | | Ref | | Ref | | Ref | |
| aARU≥550 IU, ARU-5<550 IU | 1.03 (0.44–2.42) | 0.94 | 1.15 (0.48–2.74) | 0.76 | 1.11 (0.39–3.18) | 0.84 | 1.30 (0.44–3.80) | 0.64 |
| aARU<550 IU, ARU-5≥550 IU | 1.24 (0.56–2.75) | 0.60 | 1.66 (0.73–3.78) | 0.23 | 1.15 (0.40–3.28) | 0.79 | 1.39 (0.47–4.10) | 0.55 |
| Both aARU and ARU-5≥550 IU | 2.84 (1.21–6.66) | 0.02 | 3.11 (1.23–7.86) | 0.02 | 2.27 (0.69–7.50) | 0.18 | 2.31 (0.64–8.25) | 0.20 |

Adjusted variables: age, male sex, initial NIHSS score, hypertension, DM, smoking, dyslipidemia, a history of previous stroke, a history of previous aspirin therapy, platelet counts, blood glucose levels, and TOAST. Primary outcome: composite of stroke, myocardial infarction, and vascular death. aARU indicates aspirin reaction unit measured 3 hours after aspirin loading; ARU, aspirin reaction unit; ARU-5, aspirin reaction unit measured on the fifth consecutive day of aspirin administration; DM, diabetes mellitus HR, hazard ratio; NIHSS, National Institutes of Health Stroke Score; TOAST, Trial of Org 10 172 in Acute Stroke Treatment.

**Table 5.** Cox Proportional Hazard Regression Analysis for Myocardial Infarction and Vascular Death

| | Myocardial Infarction* | | | | Vascular Death* | | | |
|---|---|---|---|---|---|---|---|---|
| | Crude HR | *P* Value | Adjusted HR | *P* Value | Crude HR | *P* Value | Adjusted HR | *P* Value |
| ARU-5 (n=805) | | 0.005 | | 0.001 | | 0.30 | | 0.46 |
| <550 IU | Ref | | Ref | | Ref | | Ref | |
| ≥550 IU | 7.27 (1.82–29.09) | | 13.64 (2.87–65.53) | | 1.53 (0.69–3.39) | | 1.38 (0.59–3.26) | |
| aARU (n=558) | | 0.70 | | 0.67 | | 0.44 | | 0.72 |
| <550 IU | Ref | | Ref | | Ref | | Ref | |
| ≥550 IU | 1.45 (0.22–9.70) | | 1.56 (0.20–11.97) | | 1.52 (0.53–4.35) | | 1.24 (0.39–3.98) | |
| ARU changes | | | | | | | | |
| Both aARU and ARU-5<550 IU (n=425) | Ref | | Ref | | Ref | | Ref | |
| aARU≥550 IU, ARU-5<550 IU (n=57) | 4.53 (0.46–45.00) | 0.84 | 4.65 (0.33–64.98) | 0.25 | 0.60 (0.11–3.44) | 0.57 | 0.65 (0.11–3.91) | 0.64 |
| aARU<550 IU, ARU-5≥550 IU (n=55) | 14.00 (2.42–80.80) | 0.003 | 40.50 (3.97–412.9) | 0.002 | 0.20 (0.01–3.85) | 0.29 | 0.25 (0.01–4.98) | 0.36 |
| Both aARU and ARU-5≥550 IU (n=21) | 4.28 (0.14–134.1) | 0.41 | 48.32 (0.78–2990.7) | 0.07 | 3.99 (1.21–13.15) | 0.02 | 2.18 (0.51–9.32) | 0.29 |

Adjusted variables: age, male sex, initial NIHSS score, hypertension, DM, smoking, dyslipidemia, a history of previous stroke, a history of previous aspirin therapy, platelet counts, blood glucose levels, and TOAST. aARU indicates aspirin reaction unit measured 3 hours after aspirin loading; ARU, aspirin reaction unit; ARU-5, aspirin reaction unit measured on the fifth consecutive day of aspirin administration; DM, diabetes mellitus; HR, hazard ratio; NIHSS, National Institutes of Health Stroke Score; TOAST, Trial of Org 10 172 in Acute Stroke Treatment.
*Uni- and multivariable Cox regression using Firth's penalized likelihood method.

The general characteristics of the patients with HAPR or NAPR are shown in Table 1. Patients with HAPR were less likely to have dyslipidemia and combined antiplatelet therapy, and had lower platelet counts and low-density lipoprotein cholesterol levels than patients with NAPR. Comparison of the patient characteristics according to their aspirin responsiveness at the time of the aARU measurement and at the status of their aARU and ARU-5 measurements are shown in Table 2.

Median follow-up duration was 365 (4–365) days. The composite of stroke, MI, and vascular death occurred in 90 patients, and the 1-year cumulative event rate in this group was 11.7%. The 1-year stroke recurrence rate was 6.3%, whereas the 1-year MI rate was 1.1% and the 1-year vascular death rate was 5.9%. The 1-year nonvascular death rate was 1.5% (n=10). Of the 49 recorded stroke events, the stroke was fatal in 4 patients and hemorrhagic in only 1 patient. At the 1-year follow-up, patients with HAPR at the time of the ARU-5 measurement were more likely to have experienced the composite of stroke, MI, and vascular death than patients with NAPR (18.8% versus 10.9%; Table 3 and Figure 1). MI was significantly more frequent in patients with HAPR than in patients with NAPR (4.7% versus 0.6%), but stroke was not more frequent in patients with HAPR than in patients with NAPR (8.8% versus 6.0%; Figure 2). Cox proportional hazard regression analysis revealed that HAPR was independently associated with an increased risk of the composite of stroke, MI, and vascular death at the time of the ARU-5 measurement (HR, 1.84; 95% CI, 1.07–3.19; Table 4 and Tables S1, S2). However, HAPR did not significantly increase the risk of stroke recurrence (HR, 1.48; 95% CI, 0.67–3.23) or vascular death (HR, 1.31; 95% CI, 0.55–3.13; Tables 4 and 5).

In the 558 patients who underwent the aARU measurement, incidence of the composite of stroke, MI, and vascular death at 1 year was not significantly different between patients with HAPR and those with NAPR. In the aARU measurement group, cumulative 1-year incidence of the composite of stroke, MI, and vascular death was 15.8% in patients with HAPR and 11.2% in patients with NAPR ($P$=0.22, by log-rank test; Figure 3A). However, patients in group 4 (patients with persistent HAPR between the aARU and ARU-5 measurements) were more likely to experience the composite of stroke, MI, and vascular events than patients in group 1 (patients with NAPR at the times of the aARU and ARU-5 measurements; Figure 3B). Cox proportional hazard regression analysis revealed that group 4 was independently associated with an increased risk of the composite of stroke, MI, and vascular death (HR, 3.11; 95% CI, 1.23–7.86; Table 4).

The factors associated with an increased risk of the primary outcome are shown in Table 6. Older age, a higher initial NIHSS score, and hypertension were linked with an increased risk of vascular events. In the subgroup analysis in which the patients were organized into groups according to whether they were previous aspirin users, previous aspirin use did not modify the effects of HAPR on outcomes (Table 7).

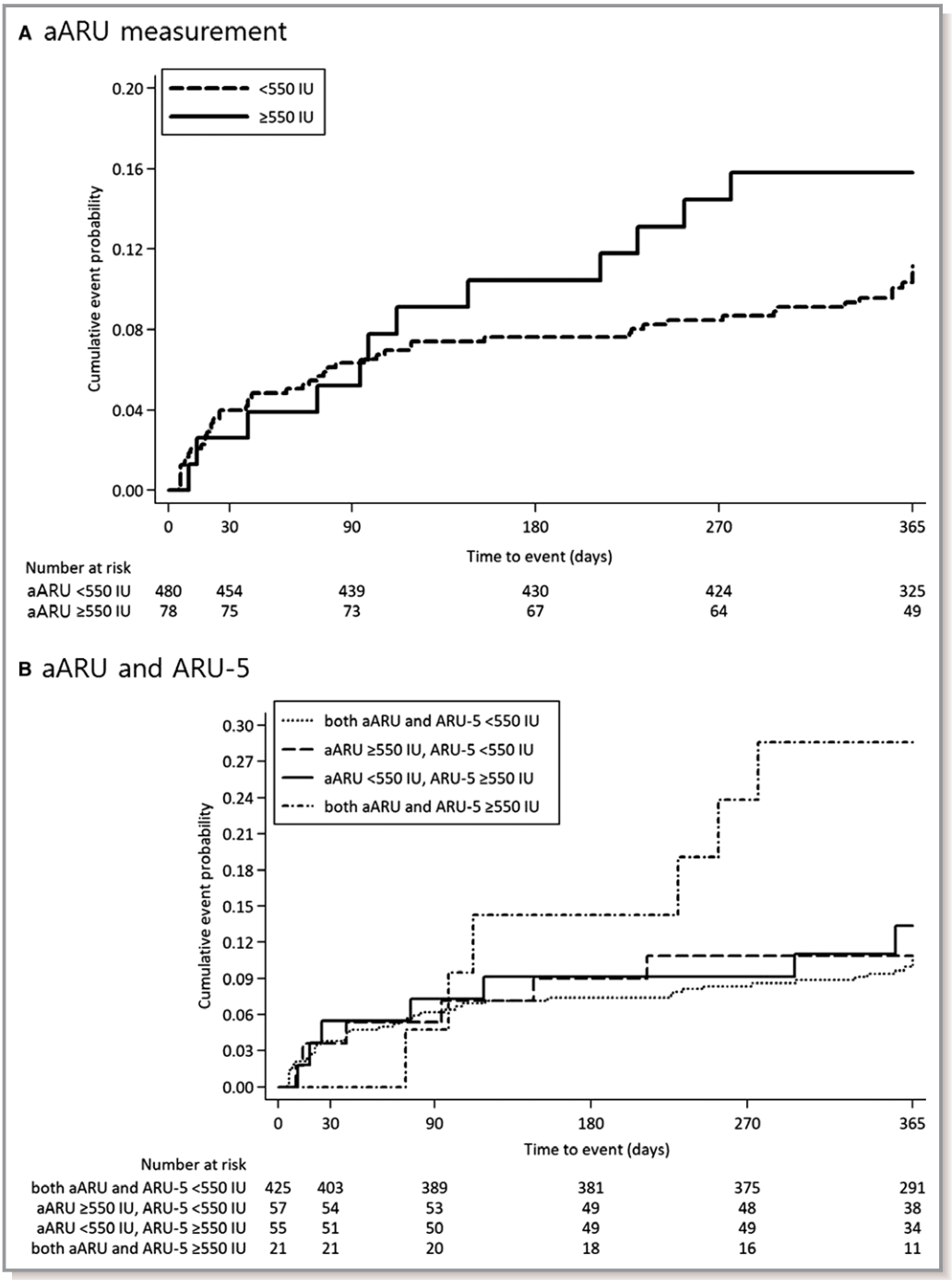


**Figure 3.** Kaplan–Meier curves showing the cumulative probability of the composite of stroke, MI, and vascular death according to whether patients had HAPR or NAPR at the time of the aARU measurement (A) and whether their HAPR status changed between the aARU and ARU-5 measurements (B). aARU indicates aspirin reaction unit measured 3 hours after aspirin loading; ARU-5, aspirin reaction unit measured on the fifth consecutive day of aspirin administration; HAPR, high on-aspirin platelet reactivity; NAPR, normal on-aspirin platelet reactivity.

## Discussion

In this prospective study on over 800 patients with acute ischemic stroke, HAPR (aspirin resistance) was associated with an increased risk of a 1-year composite of stroke, MI, and vascular death compared with NAPR after 5 consecutive days of aspirin therapy. We observed a nonsignificant trend showing that the risk of stroke was increased by 48% in patients with

**Table 6.** Cox Proportional Hazard Regression Analysis for the Primary Outcome

| | Analysis 1 | | Analysis 2 | | Analysis 3 | |
|---|---|---|---|---|---|---|
| | HR (95% CI) | *P* Value | HR (95% CI) | *P* Value | HR (95% CI) | *P* Value |
| Age, y | 1.03 (1.00–1.06) | 0.04 | 1.03 (1.00–1.06) | 0.03 | 1.04 (1.02–1.06) | 0.0004 |
| Male | 1.11 (0.62–1.98) | 0.72 | 1.03 (0.58–1.83) | 0.92 | 1.46 (0.88–2.42) | 0.14 |
| NIHSS | 1.08 (1.00–1.17) | 0.047 | 1.07 (0.99–1.15) | 0.08 | 1.04 (0.99–1.09) | 0.13 |
| Hypertension | 1.86 (1.01–3.43) | 0.046 | 1.77 (0.97–3.25) | 0.06 | 1.76 (1.08–2.89) | 0.02 |
| DM | 1.26 (0.66–2.39) | 0.47 | 1.21 (0.64–2.26) | 0.56 | 1.19 (0.71–2.01) | 0.51 |
| Dyslipidemia | 0.40 (0.14–1.11) | 0.08 | 0.38 (0.14–1.05) | 0.06 | 0.65 (0.32–1.32) | 0.23 |
| Smoking | 0.78 (0.41–1.51) | 0.47 | 0.83 (0.43–1.58) | 0.57 | 0.84 (0.50–1.41) | 0.52 |
| Previous stroke | 0.80 (0.41–1.58) | 0.52 | 0.83 (0.43–1.63) | 0.59 | 1.06 (0.62–1.83) | 0.83 |
| Previous aspirin use | 1.40 (0.80–2.45) | 0.24 | 1.37 (0.79–2.38) | 0.27 | 1.30 (0.81–2.07) | 0.27 |
| Platelet count | 1.17 (0.77–1.75) | 0.46 | 1.09 (0.72–1.64) | 0.69 | 1.17 (0.84–1.63) | 0.35 |
| Initial blood glucose | 1.20 (0.76–1.88) | 0.44 | 1.23 (0.79–1.94) | 0.36 | 1.07 (0.73–1.58) | 0.72 |
| TOAST | | | | | | |
| LAA | 1.24 (0.68–2.24) | 0.48 | 1.18 (0.65–2.12) | 0.59 | 1.23 (0.76–2.00) | 0.41 |
| SVO | 0.43 (0.17–1.08) | 0.07 | 0.43 (0.18–1.07) | 0.07 | 0.62 (0.30–1.29) | 0.20 |
| UD | Ref | | | | Ref | |
| ARU changes | | | NA | | NA | |
| Both aARU and ARU-5<550 IU | Ref | | | | | |
| aARU≥550 IU, ARU-5<550 IU | 1.15 (0.48–2.74) | 0.76 | | | | |
| aARU<550 IU, ARU-5≥550 IU | 1.66 (0.73–3.78) | 0.23 | | | | |
| Both aARU and ARU-5≥550 IU | 3.11 (1.23–7.86) | 0.02 | | | | |
| aARU≥550 IU | NA | | 1.55 (0.81–2.98) | 0.19 | NA | |
| ARU-5≥550 IU | NA | | | | 1.84 (1.07–3.19) | 0.03 |

Primary outcome: composite of stroke, myocardial infarction, and vascular death. Model 1: adjusted variables including age, male, initial NIHSS, hypertension, diabetes mellitus, dyslipidemia, smoking, previous stroke, prior aspirin use, platelet counts, blood glucose, TOAST classification, and the ARU changes (4 groups). Model 2: adjusted variables including age, male, initial NIHSS, hypertension, diabetes mellitus, dyslipidemia, smoking, previous stroke, prior aspirin use, platelet counts, blood glucose, TOAST classification, and aARU≥550 IU. Model 3: adjusted variables including age, male, initial NIHSS, hypertension, diabetes mellitus, dyslipidemia, smoking, previous stroke, prior aspirin use, platelet counts, blood glucose, TOAST classification, and ARU-5≥550 IU. aARU indicates aspirin reaction unit measured 3 hours after aspirin loading; ARU, aspirin reaction unit; ARU-5, aspirin reaction unit measured on the fifth consecutive day of aspirin administration; DM, diabetes mellitus; HR, hazard ratio; LAA, large artery atherosclerosis; NA, not applicable; NIHSS, National Institutes of Health Stroke Score; SVO, small vessel occlusion; TOAST, Trial of Org 10 172 in Acute Stroke Treatment; UD, undetermined etiology.

HAPR compared with patients with NAPR. More interestingly, persistent HAPR status at the times of the aARU and ARU-5 measurements was substantially associated with an increased risk of vascular events in the post-hoc analysis. The strength of this study was that it thoroughly explored the clinical implications of acute platelet reactivity to aspirin using both single and serial ARU measurements. The results suggest that HAPR during the acute stage of ischemic stroke could increase the risk of subsequent vascular events, and that serial ARU measurements may identify patients with acute ischemic stroke who are at a higher risk for vascular events.

Our study expands upon the findings of previous studies that showed that biological aspirin resistance increases the risk of subsequent vascular events in acute ischemic stroke.[6,13] Consistent with the findings of our study, a previous study showed that HAPR on days 7 to 10 after aspirin administration may be related to a 3.2-fold greater risk of subsequent vascular events in acute ischemic stroke.[13] Given that aspirin is the only antiplatelet medication whose use is recommended during the acute periods of ischemic stroke, as stated in the current guidelines,[14] the early postaspirin treatment ARU measurement may be important for determining the early response to aspirin and for predicting the risk of subsequent vascular events in acute ischemic stroke. HAPR at the time of the ARU-5 measurement was associated with an 84% increase in the risk of 1-year vascular events in the current study. Although HAPR upon the aARU measurement was not significantly associated with the risk of subsequent vascular events, a 55% increase in the risk of vascular events was observed. In a previous study, HAPR at the time of the aARU measurement was associated with new ischemic lesions,[12] which have been linked to subsequent ischemic stroke.[15]

**Table 7.** Subgroup Analysis According to Previous Aspirin Use

| | Composite of Stroke, Myocardial Infarction, and Vascular Death | | | | | |
|---|---|---|---|---|---|---|
| | Crude HR | *P* Value | $P_{interaction}$ | Adjusted HR | *P* Value | $P_{interaction}$ |
| Previous aspirin users (N=180) | | | 0.31 | | | 0.62 |
| ARU-5<550 IU | Ref | | | Ref | | |
| ARU-5≥550 IU | 2.58 (1.04–6.40) | 0.04 | | 2.25 (0.89–5.68) | 0.09 | |
| Naive aspirin users (N=625) | | | | | | |
| ARU-5<550 IU | Ref | | | Ref | | |
| ARU-5≥550 IU | 1.46 (0.76–2.79) | 0.26 | | 1.67 (0.85–3.29) | 0.14 | |

Adjusted variables including age, male, initial NIHSS, hypertension, diabetes mellitus, dyslipidemia, smoking, previous stroke, prior aspirin use, platelet counts, blood glucose, and TOAST classification. $P_{interaction}$: *P* for interaction effect between previous aspirin and HAPR. ARU-5 indicates aspirin reaction unit measured on the fifth consecutive day of aspirin administration; HAPR, high on-aspirin platelet reactivity; HR, hazard ratio; NIHSS, National Institutes of Health Stroke Score; TOAST, Trial of Org 10 172 in Acute Stroke Treatment.

Aspirin is important in the immediate management of acute ischemic stroke. Rothwell et al found that aspirin is the key intervention for reducing the risk of early stroke recurrence after transient ischemic attack or minor stroke. The treatment is most beneficial if administered within 2 weeks of stroke onset.[3] Therefore, HAPR in response to early aspirin treatment after acute ischemic stroke has important clinical implications. Consistency with respect to the clinical implications of aspirin resistance has been observed.[5,16,17] However, the results of several previous studies did not support the performance of antiplatelet function testing, because antiplatelet therapy modification was not associated with better outcomes in these investigations.[18,19] These studies were limited because they were not studies regarding acute ischemic stroke[18]; they were retrospective studies, and they arbitrarily chose to perform the timing of platelet function testing.[19] Therefore, additional studies are warranted to determine the effects of modifying antiplatelet regimens based on the acute ARU measurement.

Our results have implications for the performance of serial ARU measurements after early aspirin treatment in acute ischemic stroke. Persistent HAPR status from aspirin loading upon admission to the 5-day aspirin treatment period was associated with a 3-fold higher risk of subsequent vascular events than persistent NAPR. Studies investigating the clinical implications of serial ARU measurements in acute ischemic stroke are rare. In a previous study, increases in platelet reactivity to aspirin over time were independently associated with early neurological deterioration in patients with acute ischemic stroke.[11] Determining the relationship between ARU measurements and the risk of vascular events may enable patients to be stratified according to their serial ARU measurement results, which may aid in the identification of patients with acute ischemic stroke who are at higher risk for future vascular events.

Despite aspirin administration for 5 consecutive days after acute ischemic stroke, 27% of patients with HAPR at the time of the aARU measurement had persistent HAPR at the time of the ARU-5 measurement. Previous studies found that adding other antiplatelet therapies to aspirin therapy was associated with a decrease in the rate of antiplatelet resistance in antiplatelet therapy-resistant patients[20,21] and had beneficial effects on the risks of ischemic events[22]; however, there were other studies with conflicting results.[23] Given that the frequency of combination antiplatelet therapy was higher in patients with NAPR than in patients with HAPR at the time of the ARU-5 measurement, patients with HAPR at the time of the acute platelet function test may be candidates for additional antiplatelet therapies.

Previous aspirin therapy did not modify the effects of aspirin resistance on outcomes in the subgroup analysis, suggesting that the clinical effects of HAPR on outcomes could be consistent in acute ischemic stroke, regardless of whether patients were previous aspirin users. Among previous aspirin users, aspirin resistance was previously shown to be independently associated with an increased risk of large infarcts or severe stroke.[24,25] Recent studies found that the addition of antiplatelet therapies or switching to new antiplatelet therapies may be better for preventing subsequent vascular events in patients who have suffered a breakthrough stroke while on aspirin.[26,27] However, HAPR was not more frequent in previous aspirin users than in aspirin-naïve patients after early aspirin treatment for acute ischemic stroke. Accordingly, aspirin resistance after acute aspirin treatment, regardless of whether aspirin therapy was administered before stroke onset, may be a target of alternative antiplatelet therapies and the focus of future clinical research on stroke prevention.

Various factors, including genetic, biological, and clinical factors, contribute to aspirin resistance.[9,28] Presumed mechanisms that may contribute to intraindividual variability in response to aspirin, as previously speculated,[10] include alternative platelet activation pathways,[29] transient changes in aspirin bioavailability,[30] or genetic polymorphisms that

contribute to a variable response to aspirin.[31] Because potential biasing factors in our study should be well controlled, the variability in the ARU measurements would be expected to be more pronounced in clinical practice.[10]

This study had several limitations. Some of these have been described previously.[11,12] The cut-off ARU value of 550 IU for aspirin resistance is commonly used, but may be arbitrary in acute ischemic stroke. Furthermore, platelet function tests for aspirin resistance have not been standardized and do not correlate well with one another.[32] The lack of a consistent protocol for the use of antiplatelet agents was another limitation of our study. After discharge, some patients might be treated with enteric-coated aspirin, whereas all patients were administered plain aspirin upon admission. Although data suggest that enteric-coated aspirin may lead to aspirin nonresponsiveness,[33,34] the clinical implications of previous findings on all outcomes need to be established. Additionally, our study had inherent limitations that are common in single-center studies with relatively small sample sizes. This study lacked the statistical power to detect important differences (or to have a broad CI) in terms of individual end points in the subgroups of aARU or ARU changes. Therefore, some findings should be interpreted with caution, and large-scale studies are warranted to draw more-conclusive answers regarding this issue. Moreover, enrolled participants had a median NIHSS score of 2 (interquartile range, 0, 5) with mostly mild stroke severity. However, minor stroke (NIHSS 0–3) was present in 62.5% of the cohort, moderate (NIHSS 4–8) in 24.4%, and severe (NIHSS >8) in 13.1%, which was similar to the results from a Korean nation-wide stroke registry.[35] In general, the proportion of individuals who experienced minor stroke was quite high for ischemic stroke than for hemorrhagic stroke. Additionally, the generalizability of our results, which were obtained by studying a portion of the genetically disparate Korean population, to other ethnic populations evaluated in other studies is limited. Elevated body mass index or body weight could be a good predictor for aspirin resistance.[36] In contrast to the results of a previous study, the body mass index was not numerically different between patients with HAPR and those with NAPR in the current study. Because of the ethnic characteristics of the patients, only a small percentage of patients had very high body weight (>90 kg; n=10; 1.2%) or obesity (body mass index >30; n=22; 2.7%).

In conclusion, the results of our study showed that HAPR at the time of early aspirin treatment may increase the 1-year risk of vascular events in acute ischemic stroke. More interestingly, persistent HAPR during the acute period of ischemic stroke was more substantially associated with an increased risk of subsequent vascular events than persistent NAPR. These results lend support to the idea that acute and serial platelet function test results have clinical implications for the risk of future vascular events in acute ischemic stroke. Additional studies are warranted to determine appropriate treatments for patients with acute ischemic stroke with HAPR.

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## Disclosures

None.

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# SUPPLEMENTAL MATERIAL

**Table S1. Cox proportional hazard regression analysis - Primary outcome**

| | Crude HR | P | P-value by Supremum Test for Proportional Hazards Assumption | Adjusted HR | P | P-value by Supremum Test for Proportional Hazards Assumption |
|---|---|---|---|---|---|---|
| ARU-5 (n=805) | | | | | | |
| <550 IU | Ref | | | Ref | | |
| ≥550 IU | 1.69 (1.00-2.87) | 0.05 | 0.14 | 1.84 (1.07-3.19) | 0.03 | 0.14 |
| aARU (n=558) | | | | | | |
| <550 IU | Ref | | | Ref | | |
| ≥550 IU | 1.47 (0.79-2.76) | 0.23 | 0.33 | 1.55 (0.81-2.98) | 0.19 | 0.29 |
| ARU changes (n=558) | | | | | | |
| Group 1 | Ref | | | Ref | | |
| Group 2 | 1.03 (0.44-2.42) | 0.94 | 0.39 | 1.15 (0.48-2.74) | 0.76 | 0.41 |
| Group 3 | 1.24 (0.56-2.75) | 0.60 | 0.88 | 1.66 (0.73-3.78) | 0.23 | 0.87 |
| Group 4 | 2.84 (1.21-6.66) | 0.02 | 0.10 | 3.11 (1.23-7.86) | 0.02 | 0.07 |

**Table S2. Cox proportional hazard regression analysis - Stroke**

| | Crude HR | P | P-value by Supremum Test for Proportional Hazards Assumption | Adjusted HR | P | P-value by Supremum Test for Proportional Hazards Assumption |
|---|---|---|---|---|---|---|
| ARU-5 (n=805) | | | | | | |
| <550 IU | Ref | | | Ref | | |
| ≥550 IU | 1.40 (0.66-2.99) | 0.26 | 0.381 | 1.48 (0.67-3.23) | 0.33 | 0.28 |
| aARU (n=558) | | | | | | |
| <550 IU | Ref | | | Ref | | |
| ≥550 IU | 1.40 (0.62-3.18) | 0.20 | 0.42 | 1.51 (0.65-3.54) | 0.34 | 0.19 |
| ARU changes (n=558) | | | | | | |
| Group 1 | Ref | | | Ref | | |
| Group 2 | 1.11 (0.39-3.18) | 0.71 | 0.84 | 1.30 (0.44-3.80) | 0.64 | 0.72 |
| Group 3 | 1.15 (0.40-3.28) | 0.37 | 0.80 | 1.39 (0.47-4.10) | 0.55 | 0.36 |
| Group 4 | 2.27 (0.69-7.50) | 0.14 | 0.18 | 2.31 (0.64-8.25) | 0.20 | 0.11 |

*Supremum Test for Proportional Hazards Assumption using Cumulative Sums of Martingale-Based Residuals
Group 1, both aARU and ARU-5<550 IU; Group 2, aARU≥550 IU and ARU-5<550 IU; Group 3, aARU<550 IU and ARU-5≥550 IU; and Group 4, both aARU and ARU-5≥550 IU
Adjusted variables: age, male, initial NIHSS, HTN, DM, smoking, dyslipidemia, prior stroke, previous aspirin, platelet count, blood glucose, and TOAST
Primary outcome: composite events of stroke, MI, and vascular death

**Figure S1. Selection of the study population.**