Effects of Endothelial Dysfunction on Residual Platelet Aggregability After Dual Antiplatelet Therapy With Aspirin and Clopidogrel in Patients With Stable Coronary Artery Disease

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Background—Dual antiplatelet therapy with aspirin and clopidogrel is widely used in patients with coronary stents. High residual platelet reactivity (high RPR) after dual antiplatelet therapy is associated with increased cardiovascular events. Endothelial function could affect platelet reactivity in vivo. We hypothesized that endothelial dysfunction could be associated with high RPR after dual antiplatelet therapy in patients with stable coronary artery disease.

Methods and Results—We screened patients with stable coronary artery disease for cytochrome P450 (CYP) 2C19 genotypes and enrolled 103 patients who lacked CYP2C19*2 or *3 loss-of-function allele to minimize the effect of this gene on high RPR. All patients received aspirin (100 mg/d) and clopidogrel (75 mg/d for long-term treatment or a loading dose of 300 mg) before the following tests. Platelet agregability was assessed as P2Y12 reaction unit using the VerifyNow System. High RPR was defined as P2Y12 reaction unit ≥230. Peripheral endothelial function was expressed as reactive hyperemia index using reactive hyperemia peripheral arterial tonometry. Fifty-three patients exhibited high RPR. High RPR patients were significantly older, had higher levels of B-type natriuretic peptide, and were predominantly hypertensive compared with non-high RPR patients. Reactive hyperemia index was significantly lower in high RPR patients (0.46±0.15) compared with non-high RPR patients (0.61±0.18; P<0.001). Linear regression analysis demonstrated significant negative correlation between reactive hyperemia index and P2Y12 reaction unit (r=−0.32; P=0.001). Multivariable logistic regression analysis identified reactive hyperemia index as an independent and significant determinant of high RPR (odds ratio, 0.55; 95% confidence interval, 0.39–0.78; P=0.001).

Conclusions—In patients with stable coronary artery disease, endothelial function was significantly impaired in high RPR patients. Endothelial dysfunction is independently correlated with high RPR after dual antiplatelet therapy.

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Key Words: antiplatelet agents ■ atherosclerosis ■ coronary artery disease ■ endothelial function ■ platelet function tests ■ platelet reactivity ■ thrombosis

Dual antiplatelet therapy (DAPT) with aspirin and clopidogrel is effective in preventing stent thrombosis and is widely used after percutaneous coronary intervention (PCI). However, the high residual platelet reactivity (high RPR) after DAPT is associated with increased cardiovascular events.1,2 Several studies have demonstrated that RPR itself is treatable and adjustable by increasing the dose of clopidogrel,4,5 although aggressive antiplatelet therapy is reported to increase the risk of hemorrhagic events6,7 and does not always prevent the occurrence of future cardiovascular events.4,5,8
WHAT IS KNOWN

- High residual platelet reactivity after dual antiplatelet therapy is associated with increased cardiovascular events.
- Patients with variants in cytochrome P450 (CYP) 2C19 have lower levels of the active metabolite of clopidogrel with less inhibition of platelet aggregation.
- Platelet aggregation is closely associated with the integrity and condition of the vascular endothelium in vivo.

WHAT THE STUDY ADDS

- Peripheral endothelial dysfunction is independently correlated with higher residual platelet reactivity after dual antiplatelet therapy with aspirin and clopidogrel in patients with stable coronary artery disease who do not carry a CYP2C19 reduced-function allele.

The VerifyNow P2Y12 assay (Ultegra rapid platelet function assay; Accurometrics Inc, San Diego, CA) has made it possible to quantify RPR clinically at bedside and identify patients with high RPR. Thus, it is currently possible to provide tailored antiplatelet therapy by using VerifyNow P2Y12 assay. Clopidogrel is a prodrug activated in the liver by cytochrome P450 (CYP) enzymes. It has been demonstrated that patients with variants in CYP2C19 have lower levels of the active metabolite of clopidogrel with less inhibition of platelet aggregation. However, the majority of Europeans and Americans do not have CYP2C19 reduced-function allele; it has been reported that high RPR cases are often observed among whites who are not carriers of CYP2C19 reduced-function allele. Thus, the CYP2C19 gene analysis was not recommended for cardiovascular risk evaluation in the statement published by the American Heart Association. At present, the precise mechanism of high RPR remains obscure, and there is a need to understand the pathogenesis of high RPR especially in patients without CYP2C19 reduced-function allele.

Platelet aggregation is closely associated with the integrity and condition of the vascular endothelium in vivo. Endothelial dysfunction could enhance high RPR through inadequate production of nitric oxide and prostacyclin, which counteract platelet aggregation. The endothelium maintains vascular homeostasis, and endothelial dysfunction is an early marker for atherosclerosis. Because PCI evokes local coronary endothelial injury and patients with coronary artery disease (CAD) usually exhibit endothelial dysfunction, evaluation of platelet aggregability and endothelial function and defining their correlation are no doubt important in CAD patients who undergo PCI. Previous clinical studies indicated that endothelial dysfunction correlated significantly with increased platelet aggregability, but the effects of coronary risk factors and genetic polymorphisms on platelet aggregability and endothelial function have not been determined. Peripheral endothelial function and the therapeutic effectiveness of DAPT can be assessed clinically by the reactive hyperemia index using reactive hyperemia peripheral arterial tonometry (RH-PAT), and by RPR, respectively. The main hypothesis tested in the present study was that peripheral endothelial dysfunction is associated with high RPR in patients with CAD free of genetic polymorphism of CYP2C19*2 or *3 loss-of-function allele.

Methods

Study Population

The study subjects were patients with stable CAD who visited Kumamoto University Hospital between August 2008 and June 2012. CAD was defined as history of angina or myocardial ischemia by stress tests coupled with coronary stenosis of >50% of the vessel diameter detected by coronary angiography or computed tomography coronary angiography scan, or history of myocardial infarction, PCI or coronary artery bypass grafting. We excluded patients with acute coronary syndrome who required emergency coronary angiography, defined as acute myocardial infarction (with or without electrocardiographic evidence of ST-segment elevation), those with unstable angina (class II or III of Braunwald classification), or patients with known allergy to clopidogrel or thrombocytopenia (platelet count <10×10⁴/μL). We also excluded patients with collagen disease, infection, severe liver dysfunction, and malignant diseases and patients treated with ticlopidine, cilostazol, and sarpogrelate, and those on hemodialysis. There were no patients treated with n-3 polyunsaturated fatty acids. We identified CYP2C19 genotypes and finally enrolled 103 patients without CYP2C19*2 or *3 loss-of-function allele to minimize the effects of genetics on high RPR (genotype analysis in online-only Data Supplement).

This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Human Ethics Review Committee of Kumamoto University Graduate School of Medicine, and a signed consent form was obtained from each subject. This study was registered at the UMIN protocol registration system with the identification number UMIN000008239.

Study Protocol

At baseline, we measured RPR, peripheral endothelial function, and conducted various laboratory tests, such as high-sensitivity C-reactive protein and B-type natriuretic peptide (BNP), in patients with stable CAD before coronary angiography (blood sampling method in the online-only Data Supplement). RPR was assessed by VerifyNow P2Y12 assay (detail of VerifyNow P2Y12 is given in the online-only Data Supplement). A high P2Y12 reaction unit (PRU) result reflects greater P2Y12-mediated reactivity. High RPR was defined as PRU ≥230. Achievement of on-treatment reactivity of 208 PRU was independently associated with a markedly lower risk of cardiovascular outcomes at 60-day follow-up in post hoc analysis of the Gauging Responsiveness with A VerifyNow assay-Impact on Thrombosis And Safety (GRAVITAS) study. We further divided the whole population into 2 groups by the value of 208 PRU: the non-high RPR group and the high RPR group to identify patients with high RPR after DAPT. Peripheral endothelial function was assessed by RH-PAT index (RHI) using RH-PAT (details of RHI and RH-PAT in the online-only Data Supplement).

All patients were under long-term aspirin treatment (100 mg/d) for >7 days. Fifty patients already received clopidogrel (75 mg/d) for >7 days, and 53 patients were treated with a loading dose of clopidogrel (300 mg) ≥24 hours and subsequent daily 75 mg before the following tests.

Statistical Analysis

The Shapiro–Wilks test was used to assess the normal distribution of continuous data. Data are expressed as mean±SD, whereas those with skewed distributions were expressed as the median value with interquartile range. Categorical data were presented as frequencies and percentages. Differences between 2 groups were tested with Fisher
exact test for categorical variables. Differences in continuous variables were analyzed by the unpaired t test or Mann–Whitney U test, as appropriate. Pearson correlation coefficient was used for evaluation of the association between RHI and the parameters of platelet aggregation (PRU). Spearman rank correlation coefficient was used if variables were not normally distributed (the association between RHI and other clinical variables). In this study, the outcome variables for the regression model were RPR values (continuous variable) for the multivariable linear regression model and the high RPR group (dichotomous variable) for the multivariable logistic regression model. Significant clinical parameters associated with PRU in simple (univariate) logistic regression analysis and several factors reported previously to affect platelet reactivity were evaluated by multivariable logistic regression analysis. We used the Kaplan–Meier method to estimate the cardiovascular event probabilities at 1340 days, and we also used the log-rank test to compare distributions of survival times between the high RPR (or high RPR) group and the non–high RPR (or non–high RPR) group. A P<0.05 denoted statistical significance. Statistical analyses were performed using SPSS version 20 (SPSS Inc, Tokyo, Japan). An expended Methods section is available in the online-only Data Supplement.

Results

Clinical Characteristics of Study Population

Table I lists the baseline characteristics of the enrolled patients. Patients of the high RPR group were significantly older, had higher prevalence of hypertension, and had higher levels of BNP compared with the non–high RPR group. RHI was significantly lower in the high RPR group (0.46±0.15) than in the non–high RPR group (0.61±0.18; P=0.001; Figure 1).

Correlation Between RHI and PRU

There was a significant negative correlation between RHI and PRU (r=−0.32; P=0.001; Figure 2) and a significant positive correlation between RHI and % platelet inhibition (r=0.35; P=0.001). BNP also correlated significantly with PRU (r=0.34; P=0.001). However, age and hypertension did not correlate with PRU. The multivariable linear regression model consisted of RHI and the variables (age, diabetes mellitus, estimated glomerular filtration rate, and Log_{10} [BNP]) that had been independently shown to impact RPR in the previous studies.18,21–26 Each of RHI and BNP was shown as the independently associated factor for RPR (Table 2).

Association Between Endothelial Function and High RPR

Table 3 shows the results of univariate and multivariable logistic regression analyses for high RPR. Simple logistic regression analysis demonstrated that age, prevalence of hypertension, angiotensin-converting enzyme inhibitor (ACE-I) or angiotensin-II receptor blocker (ARB) use, BNP, and RHI were significantly associated with the presence of high RPR. RHI (odds ratio, 0.55; 95% confidence interval, 0.39–0.78; P=0.001) and ACE-I or ARB use (odds ratio, 3.22, 95% confidence interval, 1.07–9.65; P=0.04) were shown as the independently associated factor for high RPR among the significant variables in simple analysis. We also performed multivariable logistic regression analysis using the factors (age, body mass index, diabetes mellitus, estimated glomerular filtration rate, and Log_{10} [fibrinogen]) that had been repeatedly reported to impact the high RPR group in several studies reported previously.18,21–26 RHI and age were identified as an independent determinant of high RPR (RHI: odds ratio; 0.61; 95% confidence interval, 0.45–0.84; P=0.002; age: odds ratio, 1.06; 95% confidence interval, 1.00–1.12; P=0.04; Table 4). RHI was not associated with high-density lipoprotein cholesterol, hemoglobin A1c, or waist circumference (high-density lipoprotein cholesterol: r=0.09, P=0.39; hemoglobin A1c: r=−0.04, P=0.66; waist circumference: r=0.02, P=0.85). RHI was significantly associated with estimated glomerular filtration rate and high-sensitivity C-reactive protein (estimated glomerular filtration rate: r=0.18, P=0.04; high-sensitivity C-reactive protein: r=−0.30, P=0.003). RHI was also significantly associated with high RPR by using the cutoff value of 208 PRU (Tables I–III in the online-only Data Supplement).

We also analyzed the association in the 2 groups regarding the duration of clopidogrel treatment: the short-term loading group (≤7 days) and the long-term group (>7 days). RHI was the significant factor for high RPR in both the short-term loading and the long-term groups by methods using 2 significant factors (Table IV–VII in the online-only Data Supplement).

Cardiovascular Events in the High RPR and Non–High RPR Groups

The data of 103 patients were available for analyzing cardiovascular events. The follow-up period was 14 to 1340 days (mean: 657 days; median: 620 days). Overall, 17 cardiovascular events were recorded during the follow-up period (Table VIII in the online-only Data Supplement). The time to first cardiovascular events in the high RPR group was nonsignificantly shorter than the non–high RPR group by Kaplan–Meier analysis (log-rank test, P=0.16; Figure I in the online-only Data Supplement). We also compared the cardiovascular outcomes by using the cutoff value of 208 PRU (Table IX in the online-only Data Supplement). The high RPR group had a nonsignificant higher probability of cardiovascular events compared with the non–high RPR group by Kaplan–Meier analysis (log-rank test: P=0.16; Figure II in the online-only Data Supplement).

In Vitro Experiments of Endothelial Function and Platelet Reactivity

Platelet reactivity of human platelet-rich plasma incubated with dysfunctional human coronary artery endothelial cells treated by nitro-L-arginine methyl ester was significantly higher than that incubated with normal human coronary artery endothelial cells without nitro-L-arginine methyl ester (Figure IIIA in the online-only Data Supplement). Incubation with human coronary artery endothelial cells treated by nitro-L-arginine methyl ester did not affect the platelet count (Figure IIIB in the online-only Data Supplement).

Discussion

This is the first study to demonstrate the significant effect of peripheral endothelial dysfunction on RPR after DAPT with aspirin and clopidogrel in patients with stable CAD who were confirmed to carry no CYP2C19 reduced-function allele, which is the most established factor for high RPR in clinical setting.10,12,27 Clinical studies reported that high RPR after DAPT is associated with increased risk for cardiovascular events.1,3 However, evidence for the association between intensive antiplatelet therapy for improvement of high RPR...
and prevention of cardiovascular events is still insufficient.4,5,8 Therefore, to study the clinical importance of the pathogenesis of high RPR, we demonstrated here that endothelial dysfunction, assessed by measuring RHI, is an important and independent determinant of high RPR after DAPT in patients with stable CAD.

Clopidogrel is a prodrug that requires biotransformation by hepatic CYP enzymes into an active metabolite.9 Various factors, including variants of CYP genes and drug interaction, are associated with high RPR.10–12 The association between high RPR after DAPT and CYP2C19 single-nucleotide polymorphisms has been thoroughly investigated, and the interactive

### Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Total (n=103)</th>
<th>Non–High RPR (n=50)</th>
<th>High RPR (n=53)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>68.2±9.2</td>
<td>66.3±9.0</td>
<td>70.0±9.2</td>
<td>0.045</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>70 (68)</td>
<td>35 (70)</td>
<td>35 (66)</td>
<td>0.68</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.7±3.5</td>
<td>24.4±3.3</td>
<td>24.9±3.6</td>
<td>0.46</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>90.6±11.2</td>
<td>89.7±8.4</td>
<td>91.4±13</td>
<td>0.47</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>46 (45)</td>
<td>19 (38)</td>
<td>27 (51)</td>
<td>0.24</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>80 (78)</td>
<td>34 (68)</td>
<td>46 (87)</td>
<td>0.03</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>87 (84)</td>
<td>41 (82)</td>
<td>46 (87)</td>
<td>0.59</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>17 (17)</td>
<td>8 (16)</td>
<td>9 (17)</td>
<td>1.00</td>
</tr>
<tr>
<td>Family history, n (%)</td>
<td>30 (29)</td>
<td>12 (24)</td>
<td>18 (34)</td>
<td>0.29</td>
</tr>
<tr>
<td>OMI, n (%)</td>
<td>37 (36)</td>
<td>15 (30)</td>
<td>22 (42)</td>
<td>0.30</td>
</tr>
<tr>
<td>Peripheral artery disease, n (%)</td>
<td>13 (13)</td>
<td>5 (10)</td>
<td>8 (15)</td>
<td>0.56</td>
</tr>
<tr>
<td>β-Blockers, n (%)</td>
<td>77 (75)</td>
<td>35 (70)</td>
<td>42 (79)</td>
<td>0.37</td>
</tr>
<tr>
<td>Statins, n (%)</td>
<td>93 (90)</td>
<td>46 (92)</td>
<td>47 (89)</td>
<td>1.00</td>
</tr>
<tr>
<td>Calcium channel blockers, n (%)</td>
<td>57 (55)</td>
<td>25 (50)</td>
<td>32 (60)</td>
<td>0.33</td>
</tr>
<tr>
<td>Nitrates, n (%)</td>
<td>18 (17)</td>
<td>5 (10)</td>
<td>13 (25)</td>
<td>0.07</td>
</tr>
<tr>
<td>Proton pump inhibitors, n (%)</td>
<td>52 (50)</td>
<td>27 (54)</td>
<td>25 (47)</td>
<td>0.56</td>
</tr>
<tr>
<td>Period of clopidogrel (&gt;7 d)</td>
<td>50 (49)</td>
<td>28 (56)</td>
<td>22 (42)</td>
<td>0.17</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>13.5±1.3</td>
<td>13.6±1.3</td>
<td>13.3±1.4</td>
<td>0.32</td>
</tr>
<tr>
<td>Platelet count, 10⁹/μL</td>
<td>20.1 [17.2–23.6]</td>
<td>20.5 [18.2–23.7]</td>
<td>18.9 [16.2–22.9]</td>
<td>0.20</td>
</tr>
<tr>
<td>eGFR, mL/min/1.73 m²</td>
<td>67.0±17.1</td>
<td>69.0±18.2</td>
<td>65.0±15.8</td>
<td>0.24</td>
</tr>
<tr>
<td>Hemoglobin A1c, %</td>
<td>6.1 [5.8–6.8]</td>
<td>6.1 [5.7–6.6]</td>
<td>6.2 [5.9–7.2]</td>
<td>0.55</td>
</tr>
<tr>
<td>Insulin, n, %</td>
<td>6 (6)</td>
<td>4 (8)</td>
<td>2 (3)</td>
<td>0.43</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>159 [133–180]</td>
<td>157 [133–180]</td>
<td>159 [130–180]</td>
<td>0.84</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dL</td>
<td>46 [37–53]</td>
<td>45 [37–53]</td>
<td>46 [37–57]</td>
<td>0.66</td>
</tr>
<tr>
<td>LDL-cholesterol, mg/dL</td>
<td>92.0 [71.0–114.8]</td>
<td>92.5 [71.0–113.0]</td>
<td>92.0 [70.0–115.0]</td>
<td>0.85</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>124 [83–152]</td>
<td>124 [83–152]</td>
<td>111 [73–156]</td>
<td>0.51</td>
</tr>
<tr>
<td>Non–HDL cholesterol, mg/dL</td>
<td>109.0 [88.0–129.0]</td>
<td>109.0 [91.0–127.0]</td>
<td>111.0 [86.0–134.0]</td>
<td>0.84</td>
</tr>
<tr>
<td>hs-Tn-T, pg/mL</td>
<td>8.7 [3.0–11.8]</td>
<td>6.9 [3.0–10.0]</td>
<td>10.0 [3.3–14.9]</td>
<td>0.23</td>
</tr>
<tr>
<td>hs-CRP, mg/L</td>
<td>0.60 [0.30–1.70]</td>
<td>0.50 [0.20–1.50]</td>
<td>0.70 [0.30–2.20]</td>
<td>0.16</td>
</tr>
<tr>
<td>BNP, pg/mL</td>
<td>37.3 [19.5–85.7]</td>
<td>29.9 [15.2–57.6]</td>
<td>49.9 [24.9–108.5]</td>
<td>0.01</td>
</tr>
<tr>
<td>Coronary lesion number</td>
<td>2.0 [1.0–2.0]</td>
<td>2.0 [1.0–2.0]</td>
<td>2.0 [1.0–2.0]</td>
<td>0.66</td>
</tr>
<tr>
<td>One-vessel disease, n (%)</td>
<td>49 (48)</td>
<td>26 (52)</td>
<td>23 (43)</td>
<td>0.43</td>
</tr>
<tr>
<td>Two-vessel disease, n (%)</td>
<td>39 (38)</td>
<td>18 (36)</td>
<td>21 (40)</td>
<td>0.01</td>
</tr>
<tr>
<td>Three-vessel disease, n (%)</td>
<td>15 (15)</td>
<td>6 (12)</td>
<td>9 (17)</td>
<td>0.84</td>
</tr>
<tr>
<td>SYNTAX score</td>
<td>11.0 [7.0–19.0]</td>
<td>11.0 [7.0–17.0]</td>
<td>11.0 [7.0–21.5]</td>
<td>0.93</td>
</tr>
<tr>
<td>P2Y12 reaction units</td>
<td>237±88</td>
<td>162±46</td>
<td>308±52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% inhibition (%)</td>
<td>30.0 [16.0–50.0]</td>
<td>50.5 [42.0–60.0]</td>
<td>16.5 [8.0–27.0]</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Data are mean±SD, median [25th to 75th percentile range], or number (%). ACE-I indicates angiotensin-converting enzyme inhibitors; ARB, angiotensin-II receptor blockers; BMI, body mass index; BNP, B-type natriuretic peptide; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; hs-Tn-T, high-sensitivity troponin T; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; OMI, old myocardial infarction; RPR, residual platelet reactivity; RHI, reactive hyperemia index; and SYNTAX score, the synergy between percutaneous coronary intervention with TAXUS and cardiac surgery score.*
The effects of drugs metabolized by CYP2C19 enzyme have been well discussed.28 The majority of Europeans and Americans lack the CYP2C19 reduced-function allele,13,14 and guidelines do not recommend genetic analysis, including CYP2C19,15,29 to estimate the cardiovascular risk after DAPT. Thus, the precise pathogenic mechanism of high RPR remains unknown, although several mechanisms have been proposed for platelet response to clopidogrel. These include CYP2C19 single-nucleotide polymorphisms, age, body mass index, diabetes mellitus, renal function, C-reactive protein, fibrinogen, and endothelial function.18,21–26 In this study, we simultaneously measured peripheral endothelial function by RHI and evaluated RPR in a population lacking CYP2C19*2 or *3 loss-of-function allele, selected specifically to avoid the effects of this gene on platelet activity. The results identified peripheral endothelial dysfunction as a significant determinant of high RPR in patients with stable CAD.

Muller et al18 reported the correlation between endothelial function and RPR using RH-PAT. In their study, all patients received long-term and relative high dose of aspirin treatment (160–325 mg) and a loading dose of clopidogrel (600 mg) ≥12 hours before RH-PAT. They did not compare RH-PAT value between high RPR and non–high RPR. Furthermore, they did not carefully assess or discuss the impacts of coronary risk factors, medications, and genetic polymorphisms on RPR. The results of our study were consistent with their data and further demonstrated a significant correlation between high RPR and lower RHI, independent of other coronary risk factors, medications, and genetic polymorphisms. In our study, high RPR was still observed in the long-term treatment with clopidogrel, and the duration of clopidogrel treatment did not affect the results. This might be because the present population was patients with stable CAD.

Patients with high RPR were significantly older, more likely hypertensive, and had higher BNP level compared with the non–high RPR group. Furthermore, RHI was significantly lower in the high RPR group than in the non–high RPR group. Multivariable logistic regression analysis demonstrated that the use of ACE-I or ARB and RHI were 2 factors that significantly correlated with high RPR. There was no significant association between ACE-I or ARB and high RPR208 in the case of using the cutoff value of 208 PRU (Table I–III in the online-only Data Supplement). This result indicated that ACE-I or ARB use was not the consistently correlated factor for high RPR208. We consider that it might be just observed as the chance phenomenon that patients of the high RPR were more frequently treated with ACE-I or ARB. Advanced age and endothelial dysfunction are independently associated with high RPR. A previous study reported that aging per se correlated with high RPR.30 However, elderly patients frequently experience bleeding complications during antiplatelet therapy. Several studies demonstrated that high RPR itself was treatable and adjustable by increasing the dose of clopidogrel4,5; however, in the clinical setting, it is practically difficult to subject elderly patients to aggressive

Table 2. Results of Multivariable Regression Analysis for P2Y12 Reaction Units

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>95% CI for B</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B SEM</td>
<td>β</td>
<td>Lower Bound</td>
<td>Upper Bound</td>
</tr>
<tr>
<td>Age, per y</td>
<td>0.914</td>
<td>0.913</td>
<td>0.093</td>
<td>−0.900</td>
</tr>
<tr>
<td>Diabetes mellitus (yes)</td>
<td>19.32</td>
<td>16.60</td>
<td>0.108</td>
<td>−13.65</td>
</tr>
<tr>
<td>eGFR, per 10 ml/min per 1.73 m²</td>
<td>−0.928</td>
<td>5.238</td>
<td>−0.017</td>
<td>−11.33</td>
</tr>
<tr>
<td>Log₁₀ (BNP), per 1.0</td>
<td>62.06</td>
<td>18.18</td>
<td>0.326</td>
<td>25.95</td>
</tr>
<tr>
<td>RHI, per 0.1</td>
<td>−14.09</td>
<td>4.635</td>
<td>−0.284</td>
<td>−23.30</td>
</tr>
</tbody>
</table>

R²=0.258. The F test for a linear relationship was 6.32 with a P<0.001. Variance inflation factor: age 1.07, diabetes mellitus 1.05, eGFR 1.18, BNP 1.12, RHI 1.07. BNP indicates B-type natriuretic peptide; CI, confidence interval; eGFR, estimated glomerular filtration rate; and RHI, reactive hyperemia index.
antiplatelet therapy during DAPT. Furthermore, in the previous studies, the intensification of antiplatelet therapy by high-dose clopidogrel according to platelet reactivity did not improve clinical outcomes. Considering together, there is a need to develop a new clinical approach rather than increasing the dose of antiplatelet medications for the treatment of high RPR in future cardiovascular practice.

Endothelial function plays important roles in the maintenance of vascular tone, thrombosis, platelet adhesion, and vasculature-blood cell homeostasis, and the impaired endothelial function reflects early atherosclerotic changes. Furthermore, atherosclerogenesis involves platelet aggregation. Chronic inflammation occurs at sites of atherosclerogenesis, which also includes recruitment of platelets to the sites of endothelial injury, and activated platelet could further evoke plaque development. Endothelial dysfunction can also predict future cardiovascular events in patients with CAD. Furthermore, most plaque disruption occurs in lesions that contain a soft, lipid-rich core covered by a thin, inflamed fibrous cap, which often accompanies dysfunctional endothelium. In patients with CAD, endothelial dysfunction would be a key component of plaque vulnerability, possibly leading to the occurrence of cardiovascular complications. Endothelial function could be improved by appropriate medications and lifestyle interventions. One previous study indicated that platelet reactivity improved after a reduction in hemoglobin A1c in patients with type 2 diabetes mellitus. Statins also improve platelet reactivity in vitro. Treatment of coronary risk factors by optimal medications, with subsequent improvement of endothelial dysfunction, could attenuate enhanced platelet aggregation and reduce cardiovascular events after PCI; however, the clinical evidence is not strong enough at present, and further investigation is encouraged. The precise molecular mechanism of endothelial dysfunction in patients with high RPR after DAPT remains unknown. Previous study demonstrated that platelet-derived nitric oxide inhibits platelet aggregation by increasing intraplatelet cGMP. Ikeda et al demonstrated that the platelet-derived nitric oxide formation correlated with the number of risk factors. Our results of in

### Table 3. Results of Logistic Regression Analysis for High RPR

<table>
<thead>
<tr>
<th>Variable</th>
<th>Simple Regression</th>
<th>Multiple Regression With Significant Factors in Simple Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, per y</td>
<td>1.05</td>
<td>1.05</td>
</tr>
<tr>
<td>Male (yes)</td>
<td>0.83</td>
<td>...</td>
</tr>
<tr>
<td>BMI, per kg/m²</td>
<td>1.05</td>
<td>1.05</td>
</tr>
<tr>
<td>Diabetes mellitus (yes)</td>
<td>1.69</td>
<td>1.69</td>
</tr>
<tr>
<td>Hypertension (yes)</td>
<td>3.09</td>
<td>3.09</td>
</tr>
<tr>
<td>Current smoker (yes)</td>
<td>1.07</td>
<td>1.07</td>
</tr>
<tr>
<td>ACE-I or ARB (yes)</td>
<td>2.47</td>
<td>2.47</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>0.86</td>
<td>0.86</td>
</tr>
<tr>
<td>eGFR, per 10 mL/min per 1.73 m²</td>
<td>0.87</td>
<td>0.87</td>
</tr>
<tr>
<td>Non-HDL-cholesterol, per 10 mg/dL</td>
<td>0.97</td>
<td>0.97</td>
</tr>
<tr>
<td>LDL-cholesterol, per 10 mg/dL</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>Log₁₀ (triglycerides/10), per 1.0</td>
<td>0.44</td>
<td>0.44</td>
</tr>
<tr>
<td>Log₁₀ (hs-Tn-T), per 1.0</td>
<td>2.03</td>
<td>2.03</td>
</tr>
<tr>
<td>Log₁₀ (BNP/10), per 1.0</td>
<td>3.38</td>
<td>3.38</td>
</tr>
<tr>
<td>Log₁₀ (hs-CRP/0.1), per 1.0</td>
<td>1.72</td>
<td>1.72</td>
</tr>
<tr>
<td>Log₁₀ (fibrinogen), per 0.1</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>LVEF, per 5%</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>RHI, per 0.1</td>
<td>0.57</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Hosmer–Lemeshow goodness-of-fit χ² was 14.7 with a P value of 0.07. Nagelkerke R² was 0.39. ACE-I indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin-II receptor blocker; BMI, body mass index; BNP, B-type natriuretic peptide; CI, confidence interval; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; high RPR, high residual platelet reactivity; hs-CRP, high-sensitivity C-reactive protein; hs-Tn-T, high-sensitivity troponin T; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; OR, odds ratio; and RHI, reactive hyperemia index.

### Table 4. Results of Multiple Logistic Regression Analysis for High RPR by the Factors That Had Been Reported to Impact RPR After Dual Antiplatelet Therapy With Aspirin and Clopidogrel in the Previous Studies

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, per y</td>
<td>1.06</td>
<td>1.00–1.12</td>
<td>0.04</td>
</tr>
<tr>
<td>BMI, per kg/m²</td>
<td>0.95</td>
<td>0.87–1.16</td>
<td>0.95</td>
</tr>
<tr>
<td>Diabetes mellitus (yes)</td>
<td>1.73</td>
<td>0.68–4.42</td>
<td>0.25</td>
</tr>
<tr>
<td>eGFR, per 10 mL/min per 1.73 m²</td>
<td>0.99</td>
<td>0.75–1.32</td>
<td>0.96</td>
</tr>
<tr>
<td>Log₁₀ (fibrinogen), per 0.1</td>
<td>1.12</td>
<td>0.73–1.72</td>
<td>0.60</td>
</tr>
<tr>
<td>RHI, per 0.1</td>
<td>0.61</td>
<td>0.45–0.83</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Hosmer–Lemeshow goodness-of-fit χ² was 10.18 with a P value of 0.25. Nagelkerke R² was 0.27. BMI indicates body mass index; CI, confidence interval; eGFR, estimated glomerular filtration rate; high RPR, high residual platelet reactivity; OR, odds ratio; and RHI, reactive hyperemia index.
vitro experiments demonstrated that endothelial cells could play a role in the nitric oxide–mediated regulation of platelet activation. A previous study also demonstrated that endothelial cell–derived nitric oxide and platelet-derived nitric oxide might be important in the regulation of platelet reactivity. However, we could not conclude the correlation between endothelial cell–derived nitric oxide and platelet-derived nitric oxide in the regulation of platelet reactivity in the present study because we did not measure nitric oxide levels in the present population. Further investigations are needed.

Study Limitations
Interpretation of the results of the present study is limited by the small sample size and single-center study. Age, prevalence of hypertension, and BNP value were different between the 2 study groups. Multivariable logistic regression analysis did not identify hypertension and BNP to be associated with high RPR. The effects of advanced age and ACE-I or ARB use could not be excluded. Because the population was small, we could not conclude the significant difference in the clinical outcomes between the non–high RPR group and the high RPR group in the present study. Further clinical trials in larger population are required.

Conclusions
In patients with stable CAD who lack the CYP2C19*2 or *3 loss-of-function allele, peripheral endothelial function was significantly impaired in those with high RPR. Endothelial dysfunction is an important and modifiable factor associated independently with high RPR after DAPT with aspirin and clopidogrel.

Acknowledgments
We thank Kazunori Morita and Hiroko Miyazaki, Division of Pharmacology and Therapeutics, Graduate School of Pharmaceutical Sciences, Kumamoto University, for their assistance in measuring CYP2C19 single-nucleotide polymorphisms.

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Disclosures
None.

References


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SUPPLEMENTAL MATERIAL

Effects of Endothelial Dysfunction on Residual Platelet Aggregability after Dual Anti-platelet Therapy with Aspirin and Clopidogrel in Stable Patients with Coronary Artery Disease

Koichiro Fujisue, MD, Seigo Sugiyama, MD, PhD, Takamichi Ono, MD, PhD, Yasushi Matsuzawa, MD, PhD, Eiichi Akiyama, MD, Koichi Sugamura, MD, PhD, Junichi Matsubara, MD, PhD, Hirofumi Kurokawa, MD, Koichi Kaikita, MD, PhD, Satomi Iwashita, MT, Hitoshi Sumida, MD, PhD, Seiji Hokimoto, MD, PhD, Kentaro Oniki, MS, Kazuko Nakagawa, MD, PhD, Kunihiro Matsui, MD, MPH, Hisao Ogawa, MD, PhD

Supplemental Methods

Definition of risk factors for cardiovascular disease and peripheral arterial disease

Risk factors for cardiovascular disease were defined as following; hypertension (>140/90 mm Hg or taking antihypertensive medication), dyslipidemia (high-density lipoprotein cholesterol <40 mg/dl, low-density lipoprotein cholesterol ≥140 mg/dl, or triglycerides >150 mg/dl or taking medication for dyslipidemia), and diabetes mellitus (symptoms of diabetes plus casual plasma glucose concentration ≥200 mg/dl, fasting plasma glucose concentration ≥126 mg/dl, 2 hours plasma glucose concentration ≥200 mg/dl during 75-g oral glucose tolerance test, or taking medication for diabetes mellitus), and current smoking (smoking within 1 year). Peripheral arterial disease (PAD) was diagnosed by previous revascularization in lower limbs, or occlusive arteries in lower limbs by imaging examinations with ankle brachial index
(ABI) <0.9 or Fontaine classification≥II. None of the enrolled patients had critical limb ischemia. In patients with ABI >1.4, further vascular ultrasound examination or other imaging examinations (computed tomographic angiography or magnetic resonance angiography) were performed to detect the presence of PAD. Coronary anatomical complexity was assessed by the synergy between percutaneous coronary intervention with TAXUS and cardiac surgery (SYNTAX) score. The detail of this algorithm has been described previously.\(^1\) The detail of this algorithm has been described previously.

**Blood sampling and measurement of residual platelet aggregability**

Early morning fasting blood samples were collected for baseline laboratory tests from the antecubital vein using standard phlebotomy techniques. Platelet function was assessed before elective coronary angiography. Blood samples for platelet function were collected using the double-syringe technique, in which the first 2 to 4 ml of blood was discarded to avoid spontaneous platelet activation. Platelet function was measured using the VerifyNow P2Y12 assay (Ultegra rapid platelet function assay; Accumetrics Inc., San Diego, CA). The protocol of this test has been described in detail previously.\(^2\) The Vacutainer was inverted 3 to 5 times for gentle mixing and sent immediately to the laboratory. For VerifyNow assay, the blood samples collected at each time point for the P2Y12 cartridge were drawn into two 1.8 ml blood collection tubes containing 3.2% sodium citrate, and analyzed after 30 min and within 2 hours of blood collection. In brief, this test measures adenosine diphosphate-induced platelet
agglutination as an increase in light transmittance and uses a proprietary algorithm to report the values in P2Y12 reaction units (PRU).

**Reactive hyperemia-peripheral arterial tonometry (RH-PAT)**

The principle of RH-PAT has been described previously.³ This test was performed in a comfortable environment in the early morning before taking any daily medication within five days prior to CAG. In brief, the device measures changes in blood volume in the distal finger that accompany pulse waves. A blood pressure cuff was placed on one upper arm, while the contralateral arm served as a control. PAT probes were placed on one finger of each hand. After a 5-minute rest period, the cuff was inflated to 60 mm Hg greater than the systolic pressure or 200 mm Hg for 5 minutes and then deflated to induce reactive hyperemia. The RH-PAT data were digitally analyzed online by a computer in an operator-independent manner (Endo-PAT2000, Itamar Medical, Caesarea, Israel, software version 3.0.4). The RH-PAT value reflects the extent of reactive hyperemia, and was calculated as the ratio of the average amplitude of PAT signal over 1 min starting 1.5 min after cuff deflation (control arm, A; occluded arm, C) divided by the average amplitude of PAT signal of a 2.5-min time period before cuff inflation (baseline) (control arm, B; occluded arm, D). Because RH-PAT ratio; (C/D)/(A/B) results have a skewed distribution, the natural logarithmic scaled RH-PAT value was calculated and expressed as reactive hyperemia index (RHI). The RHI was derived from the following
equation: \( RHI = \ln\{[\text{RH-PAT ratio}] \times [0.226 \ln(\text{baseline}) - 0.2]\} \). Previous studies demonstrated the determination of reproducibility of RH-PAT examination.\(^4,5\)

**Genotype analysis**

Genomic DNA was isolated from whole blood using the DNA Extractor WB kit (Wako Pure Chemical Industries, Osaka, Japan) using the protocol of Richards et al.\(^6\) with some modification. Polymerase chain reaction restriction fragment length polymorphisms for CYP2C19*2 (681G>A) and CYP2C19*3 (636G>A) were performed as described previously.\(^7,8\) CYP2C19*2 and *3 are considered to account for >99% of alleles generating the null-activity enzyme protein in the Japanese population.\(^8\) We excluded carriers of one loss-of-function allele (*1/*2, *1/*3) and two loss-of-function alleles (*2/*2, *2/*3, *3/*3).

**Follow-up study of cardiovascular events**

After the assessment of P2Y12 reaction unit (PRU), we compared the outcome at 1340 days between the non-High-RPR and the High-RPR group. The endpoint was a composite of cardiovascular death, nonfatal myocardial infarction, unstable angina pectoris, nonfatal ischemic stroke, hospitalization for heart failure decompensation, or coronary revascularization. Cardiovascular events were ascertained from a review of medical records and confirmed by direct contact with the patients, their families, and physicians. Myocardial infarction was defined as the
detection of rise or fall of cardiac biomarkers (plasma creatine kinase-MB or cardiac troponin-T) above the 99th percentile of the upper limit of normal together with evidence of myocardial ischemia with at least one of the following, symptom, ECG changes (new ST-T changes, left bundle branch block, or pathological Q wave) or imaging evidence of new loss of viable myocardium or new regional wall motion abnormality. Unstable angina pectoris was defined as new or accelerating symptoms of myocardial ischemia accompanied by new ischemic ST-T-wave changes. Ischemic stroke was defined as based on both of neurological symptoms and radiological evidence excluding intracranial hemorrhage. Hospitalization for heart failure decompensation was defined if the patient was admitted with symptoms typical of heart failure and had objective signs of worsening heart failure requiring intravenous drug administration.

In vitro experiment

Blood samples and preparation of platelet rich plasma

Venous blood was obtained from fasted healthy volunteers who had not taken any medication for the prior 2 weeks using a glass tube containing a solution of 0.38% sodium citrate. Platelet-rich plasma was prepared by centrifugation at 900 rpm at room temperature for 15 min. Platelet-poor plasma was obtained further centrifugation of platelet-rich plasma for 10 min at 3000 rpm at room temperature.
Human Endothelial Cell Culture

Human coronary artery endothelial cells (HCAECs; Lonza Walkersville, Inc.,
Walkersville, MD) were cultured in EGM-2MV medium (Lonza Walkersville, Inc.) at
37°C in a humidified atmosphere with 5% CO₂. Cells were grown in twelve-well
plates to 80-85% confluence. HCAECs were pretreated with 1 mmol/L
Nitro-L-arginine methyl ester (L-NAME) (Sigma-Aldrich Co.) or vehicle for 12 hours.
Then 1 mL of PBS was poured onto the plates, gently shaken, and sucked off the PBS
with a pipette to remove the L-NAME. This washing procedure was performed three
times. The platelet-rich plasma was overlayed on the treated HCAECs
(L-NAME-treated and vehicle-treated cells) and it was further incubated with the
HCAECs for 1-hr. After the incubation, the platelet-rich plasma was gently collected
for the examination of platelet aggregation with adenosine diphosphate.

Measurement of platelet aggregation in the in vitro experiments

Aggregation in platelet-rich plasma induced by 5 µmol/L adenosine
diphosphate (ADP; Chrono-Log, Tokyo, Japan) was measured using a light
transmission aggregometer (MCM HEMA TRACER 313; PAM12C, LMS Inc., Japan),
where the degree of light transmission of platelet-rich plasma was defined as 0% of the
aggregation rate, and the cognitive platelet-poor plasma as 100%. The modified
platelet reactivity was defined as the area under the platelet aggregation curve during
10 min test time, and was expressed as the aggregation response over the measured time (aggregation units minute; AU min).

Statistical analysis

We determined the independent correlation between endothelial function assessed by RHI and RPR values (or the High-RPR group) among the variables that had been significantly shown to impact on RPR in the previous studies.\textsuperscript{10-16} The multivariable linear regression model was consisted of RHI and the variables (age, diabetes mellitus, estimated glomerular filtration rate (eGFR), and \( \log_{10} [\text{BNP}] \)) that had been independently shown to impact on RPR in the previous studies.\textsuperscript{10-16} The multivariable logistic regression analysis was performed to evaluate the independently associated factor for the High-RPR group with the significant variable in simple analysis. Furthermore, the multivariable logistic regression analysis using the significant factors (age, BMI, diabetes mellitus, eGFR, and \( \log_{10} [\text{fibrinogen}] \)) that had been repeatedly reported to impact on the High-RPR group in the previous several studies\textsuperscript{10-16} was determined. \( R^2 \) for the multivariate linear regression and Nagelkerke’s \( R^2 \) for the multivariate logistic regression analyses were calculated to show predictive power of the models. To assess model calibration, the Hosmer-Lemeshow statistics for multivariable logistic regression was applied. F-statistics and variance inflation factor were evaluated in the multivariable linear regression analysis.
Supplemental References


H. Incremental prognostic significance of peripheral endothelial dysfunction in patients with heart failure with normal left ventricular ejection fraction. *J Am Coll Cardiol.* 2012;60:1778-1786


<table>
<thead>
<tr>
<th></th>
<th>Total (n=103)</th>
<th>non-High-RPR&lt;sub&gt;208&lt;/sub&gt; (n=41)</th>
<th>High-RPR&lt;sub&gt;208&lt;/sub&gt; (n=62)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>68.2±9.2</td>
<td>66.3±9.4</td>
<td>69.4±9.0</td>
<td>0.10</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>70 (68)</td>
<td>28 (68)</td>
<td>42 (68)</td>
<td>1.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.7±3.5</td>
<td>24.9±3.2</td>
<td>24.5±3.7</td>
<td>0.58</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>90.6±11.2</td>
<td>91.0±8.3</td>
<td>90.4±12.7</td>
<td>0.78</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>46 (45)</td>
<td>15 (37)</td>
<td>31 (50)</td>
<td>0.23</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>80 (78)</td>
<td>25 (61)</td>
<td>55 (89)</td>
<td>0.01</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>87 (84)</td>
<td>36 (89)</td>
<td>51 (82)</td>
<td>0.58</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>17 (17)</td>
<td>7 (17)</td>
<td>10 (16)</td>
<td>1.0</td>
</tr>
<tr>
<td>Family history, n (%)</td>
<td>30 (29)</td>
<td>10 (24)</td>
<td>20 (32)</td>
<td>0.51</td>
</tr>
<tr>
<td>OMI, n (%)</td>
<td>37 (36)</td>
<td>12 (29)</td>
<td>25 (40)</td>
<td>0.30</td>
</tr>
<tr>
<td>Peripheral artery disease, n (%)</td>
<td>13 (13)</td>
<td>3 (7)</td>
<td>10 (16)</td>
<td>0.24</td>
</tr>
<tr>
<td>Beta blockers, n (%)</td>
<td>77 (75)</td>
<td>28 (68)</td>
<td>49 (79)</td>
<td>0.25</td>
</tr>
<tr>
<td>Statins, n (%)</td>
<td>93 (90)</td>
<td>39 (95)</td>
<td>54 (87)</td>
<td>0.31</td>
</tr>
<tr>
<td>ACE-I or ARB, n (%)</td>
<td>70 (68)</td>
<td>23 (56)</td>
<td>47 (76)</td>
<td>0.05</td>
</tr>
<tr>
<td>Calcium channel blockers, n (%)</td>
<td>57 (55)</td>
<td>22 (54)</td>
<td>35 (56)</td>
<td>0.84</td>
</tr>
<tr>
<td>Nitrates, n (%)</td>
<td>18 (17)</td>
<td>4 (10)</td>
<td>14 (23)</td>
<td>0.12</td>
</tr>
<tr>
<td>Proton pomp inhibitors, n (%)</td>
<td>52 (50)</td>
<td>21 (51)</td>
<td>31 (76)</td>
<td>1.0</td>
</tr>
<tr>
<td>Period of Clopidogrel (&gt;7 days)</td>
<td>50 (49)</td>
<td>17 (41)</td>
<td>36 (58)</td>
<td>0.11</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>67.0±17.1</td>
<td>69.0±18.2</td>
<td>65.0±15.8</td>
<td>0.24</td>
</tr>
<tr>
<td>Hemoglobin A1c (%)</td>
<td>6.1 [5.8–6.8]</td>
<td>6.0 [5.7–6.4]</td>
<td>6.2 [5.9–6.9]</td>
<td>0.38</td>
</tr>
<tr>
<td>Insulin, n (%)</td>
<td>6 (6)</td>
<td>3 (7)</td>
<td>3 (5)</td>
<td>0.68</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>159 [133–180]</td>
<td>159 [133–181]</td>
<td>158 [131–179]</td>
<td>0.90</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>46 [37–53]</td>
<td>44 [37–53]</td>
<td>46 [36–57]</td>
<td>0.81</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>92.0 [71.0–114.8]</td>
<td>93.0 [72.5–112.5]</td>
<td>90.0 [70.0–115.0]</td>
<td>0.63</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>118 [77–155]</td>
<td>122 [82–146]</td>
<td>116 [73–165]</td>
<td>0.94</td>
</tr>
<tr>
<td>Non-HDL cholesterol (mg/dl)</td>
<td>109.0 [88.0–129.0]</td>
<td>110.5 [91.0–131.0]</td>
<td>102.5 [86.0–134.0]</td>
<td>0.72</td>
</tr>
<tr>
<td>Hs Tn-T (pg/ml)</td>
<td>8.7 [3.0–11.8]</td>
<td>6.8 [3.0–10.0]</td>
<td>10.0 [3.6–16.9]</td>
<td>0.18</td>
</tr>
<tr>
<td>Hs CRP (mg/l)</td>
<td>0.60 [0.30–1.70]</td>
<td>0.50 [0.20–1.20]</td>
<td>0.80 [0.30–2.60]</td>
<td>0.41</td>
</tr>
<tr>
<td>BNP (pg/ml)</td>
<td>37.3 [19.5–85.7]</td>
<td>27.2 [9.3–50.0]</td>
<td>49.9 [24.9–108.5]</td>
<td>0.047</td>
</tr>
<tr>
<td>Coronary lesion number</td>
<td>2.0 [1.0–2.0]</td>
<td>1.0 [1.0–2.0]</td>
<td>2.0 [1.0–2.0]</td>
<td>0.40</td>
</tr>
<tr>
<td>One vessel disease, n (%)</td>
<td>49 (48)</td>
<td>23 (56)</td>
<td>26 (62)</td>
<td></td>
</tr>
<tr>
<td>Two vessel disease, n (%)</td>
<td>39 (38)</td>
<td>14 (34)</td>
<td>25 (40)</td>
<td></td>
</tr>
<tr>
<td>Three vessel disease, n (%)</td>
<td>15 (15)</td>
<td>4 (10)</td>
<td>11 (18)</td>
<td></td>
</tr>
<tr>
<td>SYNTAX socre</td>
<td>11.0 [7.0–19.0]</td>
<td>11.0 [6.0–17.0]</td>
<td>11.5 [7.0–20.0]</td>
<td>0.87</td>
</tr>
<tr>
<td>RHI</td>
<td>0.53±0.18</td>
<td>0.63±0.18</td>
<td>0.47±0.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P2Y12 reaction units</td>
<td>237±88</td>
<td>150±41</td>
<td>295±57</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% inhibition (%)</td>
<td>30.0 [16.0–50.0]</td>
<td>54.0 [45.0–67.0]</td>
<td>19.0 [12.0–31.0]</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

12
Data are mean±SD, median [25th to 75th percentile range], or number (%). RPR indicates residual platelet reactivity; BMI, body mass index; OMI, old myocardial infarction; ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Hs Tn-T, high-sensitivity troponin T; Hs CRP, C-reactive protein; BNP, B-type natriuretic peptide; LVEF, left ventricular ejection fraction; RHI, reactive hyperemia index; and SYNTAX score, the synergy between percutaneous coronary intervention with TAXUS and cardiac surgery score.

Supplemental Table 2. Results of logistic regression analysis for High-RPR (>208).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Simple Regression</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Multiple Regression with significant factors in simple analysis</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95%-CI</td>
<td>p value</td>
<td>OR</td>
<td>95%-CI</td>
<td>p value</td>
<td>OR</td>
<td>95%-CI</td>
<td>p value</td>
</tr>
<tr>
<td>Age (per year)</td>
<td>1.04</td>
<td>0.99–1.09</td>
<td>0.10</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Male (yes)</td>
<td>0.98</td>
<td>0.42–2.27</td>
<td>0.95</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BMI (per kg/m²)</td>
<td>0.97</td>
<td>0.86–1.09</td>
<td>0.58</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Diabetes mellitus (yes)</td>
<td>1.73</td>
<td>0.77–3.89</td>
<td>0.18</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hypertension (yes)</td>
<td>5.03</td>
<td>1.84–13.8</td>
<td>0.002</td>
<td>3.61</td>
<td>1.03–12.6</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker (yes)</td>
<td>0.93</td>
<td>0.32–2.69</td>
<td>0.90</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ACE-I or ARB (yes)</td>
<td>2.45</td>
<td>1.05–5.72</td>
<td>0.05</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Platelet count (per 10⁹/µl)</td>
<td>0.91</td>
<td>0.83–0.99</td>
<td>0.03</td>
<td>0.85</td>
<td>0.75–0.97</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR (per 10 ml/min/1.73 m²)</td>
<td>0.84</td>
<td>0.66–1.06</td>
<td>0.15</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Non-HDL-cholesterol (per 10 mg/dl)</td>
<td>0.95</td>
<td>0.84–1.07</td>
<td>0.41</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>LDL-cholesterol (per 10 mg/dl)</td>
<td>0.96</td>
<td>0.84–1.09</td>
<td>0.53</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Log₁₀ [Triglycerides/10] (per 1.0)</td>
<td>0.68</td>
<td>0.11–4.14</td>
<td>0.67</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Log₁₀ [Hs Tn-T] (per 1.0)</td>
<td>2.41</td>
<td>0.74–7.88</td>
<td>0.15</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Log₁₀ [BNP/10] (per 1.0)</td>
<td>5.38</td>
<td>1.93–15.0</td>
<td>0.01</td>
<td>7.09</td>
<td>2.04–24.7</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log₁₀ [Hs CRP/0.1] (per 1.0)</td>
<td>2.26</td>
<td>1.01–5.04</td>
<td>0.05</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Log₁₀ [Fibrinogen] (per 0.1)</td>
<td>1.32</td>
<td>0.90–1.95</td>
<td>0.16</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>LVEF (per 5 %)</td>
<td>0.90</td>
<td>0.73–1.11</td>
<td>0.33</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>RHI (per 0.1)</td>
<td>0.57</td>
<td>0.43–0.76</td>
<td>&lt;0.001</td>
<td>0.54</td>
<td>0.38–0.78</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Hosmer-Lemeshow goodness of fit chi-square was 10.57 with a p value of 0.23.
Nagelkerke R² was 0.47.
OR indicates odds ratio; and CI, confidence interval. See Table 1 for other abbreviations.
Supplemental Table 3. Results of multiple logistic regression analysis for High-RPR (>208) by the factors that had been reported to impact on RPR after dual anti-platelet therapy with aspirin and clopidogrel in the previous studies.

<table>
<thead>
<tr>
<th>variable</th>
<th>Multiple Regression with significant factors in previous studies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
</tr>
<tr>
<td>Age (per year)</td>
<td>1.02</td>
</tr>
<tr>
<td>BMI (per kg/m$^2$)</td>
<td>0.91</td>
</tr>
<tr>
<td>Diabetes mellitus (yes)</td>
<td>2.05</td>
</tr>
<tr>
<td>eGFR (per 10 ml/min/1.73 m$^2$)</td>
<td>0.88</td>
</tr>
<tr>
<td>Log$_{10}$ [Fibrinogen] (per 0.1)</td>
<td>1.09</td>
</tr>
<tr>
<td>RHI (per 0.1)</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Hosmer-Lemeshow goodness of fit chi-square was 12.51 with a p value of 0.13.
Nagelkerke $R^2$ was 0.28.
See Supplemental Tables 1 and 2 for abbreviations.
### Supplemental Table 4. Baseline characteristics in the short-term (≤ 7 days) treatment of clopidogrel group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n=53)</th>
<th>non-High-RPR (n=22)</th>
<th>High-RPR (n=31)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67.6±9.1</td>
<td>65.7±9.2</td>
<td>68.9±8.7</td>
<td>0.21</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>37 (70)</td>
<td>16 (72)</td>
<td>21 (68)</td>
<td>0.77</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.0±3.7</td>
<td>24.6±3.7</td>
<td>25.2±3.8</td>
<td>0.53</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>92.0±8.7</td>
<td>89.6±8.6</td>
<td>93.6±8.6</td>
<td>0.11</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>26 (49)</td>
<td>8 (36)</td>
<td>18 (58)</td>
<td>0.17</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>41 (77)</td>
<td>14 (64)</td>
<td>27 (87)</td>
<td>0.06</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>48 (91)</td>
<td>20 (91)</td>
<td>28 (90)</td>
<td>1.0</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>11 (21)</td>
<td>5 (23)</td>
<td>6 (19)</td>
<td>1.0</td>
</tr>
<tr>
<td>Family history, n (%)</td>
<td>13 (25)</td>
<td>5 (23)</td>
<td>8 (26)</td>
<td>1.0</td>
</tr>
<tr>
<td>OMI, n (%)</td>
<td>17 (32)</td>
<td>5 (23)</td>
<td>12 (39)</td>
<td>0.25</td>
</tr>
<tr>
<td>Peripheral artery disease, n (%)</td>
<td>7 (13)</td>
<td>3 (14)</td>
<td>4 (13)</td>
<td>1.0</td>
</tr>
<tr>
<td>Beta blockers, n (%)</td>
<td>36 (68)</td>
<td>13 (59)</td>
<td>23 (74)</td>
<td>0.37</td>
</tr>
<tr>
<td>Statins, n (%)</td>
<td>47 (89)</td>
<td>19 (86)</td>
<td>28 (90)</td>
<td>0.64</td>
</tr>
<tr>
<td>ACE-I or ARB, n (%)</td>
<td>38 (72)</td>
<td>13 (59)</td>
<td>25 (81)</td>
<td>0.12</td>
</tr>
<tr>
<td>Calcium channel blockers, n (%)</td>
<td>27 (51)</td>
<td>10 (45)</td>
<td>17 (55)</td>
<td>0.58</td>
</tr>
<tr>
<td>Nitrates, n (%)</td>
<td>9 (17)</td>
<td>1 (5)</td>
<td>8 (26)</td>
<td>0.06</td>
</tr>
<tr>
<td>Proton pomp inhibitors, n (%)</td>
<td>23 (43)</td>
<td>12 (55)</td>
<td>11 (35)</td>
<td>0.26</td>
</tr>
<tr>
<td>Platelet count (10⁹/µl)</td>
<td>19.6 [17.3–23.5]</td>
<td>22.3 [19.6–24.0]</td>
<td>18.5 [16.7–21.7]</td>
<td>0.01</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>68.4±16.2</td>
<td>68.2±17.0</td>
<td>68.5±15.8</td>
<td>0.95</td>
</tr>
<tr>
<td>Hemoglobin A1c (%)</td>
<td>6.1 [5.8–6.9]</td>
<td>6.0 [5.7–6.4]</td>
<td>6.3 [5.9–7.4]</td>
<td>0.41</td>
</tr>
<tr>
<td>Insulin, n (%)</td>
<td>2 (4)</td>
<td>1 (5)</td>
<td>1 (3)</td>
<td>1.0</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>165 [140–194]</td>
<td>174 [143–205]</td>
<td>162 [140–181]</td>
<td>0.57</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>48 [37–64]</td>
<td>47 [37–64]</td>
<td>48 [38–64]</td>
<td>0.87</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>96.0 [73.0–123.0]</td>
<td>106.0 [73.0–135.0]</td>
<td>91.0 [71.5–111.0]</td>
<td>0.24</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>121 [82–154]</td>
<td>115 [85–146]</td>
<td>123 [88–163]</td>
<td>0.87</td>
</tr>
<tr>
<td>Non-HDL cholesterol (mg/dl)</td>
<td>113.5 [91.0–136.5]</td>
<td>121.0 [94.0–143.0]</td>
<td>111.0 [88.0–130.5]</td>
<td>0.57</td>
</tr>
<tr>
<td>Hs CRP (mg/l)</td>
<td>0.60 [0.30–1.50]</td>
<td>0.60 [0.30–1.50]</td>
<td>0.60 [0.30–1.50]</td>
<td>0.93</td>
</tr>
<tr>
<td>BNP (pg/ml)</td>
<td>33.6 [19.7–100.2]</td>
<td>30.7 [13.5–52.0]</td>
<td>34.8 [24.0–108.5]</td>
<td>0.91</td>
</tr>
<tr>
<td>Coronary lesion number</td>
<td>1.0 [1.0–2.0]</td>
<td>1.0 [1.0–2.0]</td>
<td>1.0 [1.0–2.0]</td>
<td>0.21</td>
</tr>
<tr>
<td>One vessel disease, n (%)</td>
<td>32 (60)</td>
<td>16 (73)</td>
<td>16 (52)</td>
<td></td>
</tr>
<tr>
<td>Two vessel disease, n (%)</td>
<td>14 (26)</td>
<td>5 (23)</td>
<td>9 (30)</td>
<td></td>
</tr>
<tr>
<td>Three vessel disease, n (%)</td>
<td>7 (13)</td>
<td>1 (5)</td>
<td>6 (19)</td>
<td></td>
</tr>
<tr>
<td>SYNTAX score</td>
<td>11.0 [6.0–17.0]</td>
<td>11.0 [7.0–17.0]</td>
<td>11.0 [6.0–18.3]</td>
<td>0.98</td>
</tr>
<tr>
<td>RHI</td>
<td>0.53±0.17</td>
<td>0.60±0.17</td>
<td>0.49±0.15</td>
<td>0.01</td>
</tr>
<tr>
<td>P2Y12 reaction units</td>
<td>251±86</td>
<td>166±46</td>
<td>310±50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% inhibition (%)</td>
<td>30.0 [14.0–46.0]</td>
<td>52.0 [41.5–63.5]</td>
<td>15.0 [0–23.0]</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Supplemental Table 5. Results of logistic regression analysis for High-RPR in the short-term (≤ 7 days) treatment of clopidogrel

<table>
<thead>
<tr>
<th>Variable</th>
<th>Simple Regression</th>
<th>Multiple Regression with significant factors in simple analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95%-CI</td>
</tr>
<tr>
<td>Age (per year)</td>
<td>1.04</td>
<td>0.98–1.11</td>
</tr>
<tr>
<td>Male (yes)</td>
<td>0.79</td>
<td>0.24–2.62</td>
</tr>
<tr>
<td>BMI (per kg/m²)</td>
<td>1.05</td>
<td>0.90–1.23</td>
</tr>
<tr>
<td>Diabetes mellitus (yes)</td>
<td>2.42</td>
<td>0.79–7.46</td>
</tr>
<tr>
<td>Hypertension (yes)</td>
<td>3.86</td>
<td>0.99–15.1</td>
</tr>
<tr>
<td>Current smoker (yes)</td>
<td>0.82</td>
<td>0.21–3.11</td>
</tr>
<tr>
<td>Platelet count (per 10^4/µl)</td>
<td>0.82</td>
<td>0.70–0.96</td>
</tr>
<tr>
<td>eGFR (per 10 ml/min/1.73 m²)</td>
<td>1.01</td>
<td>0.72–1.42</td>
</tr>
<tr>
<td>Non-HDL-cholesterol (per 10 mg/dl)</td>
<td>0.92</td>
<td>0.78–1.09</td>
</tr>
<tr>
<td>LDL-cholesterol (per 10 mg/dl)</td>
<td>0.90</td>
<td>0.75–1.07</td>
</tr>
<tr>
<td>Log_{10} [Triglycerides/10] (per 1.0)</td>
<td>1.63</td>
<td>0.12–23.25</td>
</tr>
<tr>
<td>Log_{10} [Hs Tn-T] (per 1.0)</td>
<td>0.77</td>
<td>0.15–4.15</td>
</tr>
<tr>
<td>Log_{10} [BNP/10] (per 1.0)</td>
<td>2.60</td>
<td>0.70–9.65</td>
</tr>
<tr>
<td>Log_{10} [Hs CRP/0.1] (per 1.0)</td>
<td>1.50</td>
<td>0.50–4.17</td>
</tr>
<tr>
<td>Log_{10} [Fibrinogen] (per 0.1)</td>
<td>0.97</td>
<td>0.57–1.64</td>
</tr>
<tr>
<td>LVEF (per 5 %)</td>
<td>1.03</td>
<td>0.77–1.38</td>
</tr>
<tr>
<td>RHI (per 0.1)</td>
<td>0.61</td>
<td>0.40–0.93</td>
</tr>
</tbody>
</table>

Hosmer-Lemeshow goodness of fit chi-square was 0.80 with a p value of 1.00.

Nagelkerke R² was 0.30.

See Supplemental Tables 1 and 2 for abbreviations.
### Supplemental Table 6. Baseline characteristics in the long-term treatment (> 7 days) of clopidogrel group

<table>
<thead>
<tr>
<th></th>
<th>Total (n=50)</th>
<th>non-High-RPR (n=28)</th>
<th>High-RPR (n=22)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>68.9±9.3</td>
<td>66.8±8.6</td>
<td>71.5±9.8</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Male, n (%)</strong></td>
<td>33 (66)</td>
<td>19 (68)</td>
<td>14 (64)</td>
<td>0.77</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>24.3±3.2</td>
<td>24.3±3.0</td>
<td>24.4±3.5</td>
<td>0.85</td>
</tr>
<tr>
<td><strong>Waist circumference (cm)</strong></td>
<td>89.1±13.2</td>
<td>89.9±8.5</td>
<td>88.3±13</td>
<td>0.68</td>
</tr>
<tr>
<td><strong>Diabetes mellitus, n (%)</strong></td>
<td>20 (40)</td>
<td>11 (39)</td>
<td>9 (41)</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Hypertension, n (%)</strong></td>
<td>39 (78)</td>
<td>20 (71)</td>
<td>19 (86)</td>
<td>0.31</td>
</tr>
<tr>
<td><strong>Dyslipidemia, n (%)</strong></td>
<td>39 (78)</td>
<td>21 (75)</td>
<td>18 (82)</td>
<td>0.73</td>
</tr>
<tr>
<td><strong>Current smoker, n (%)</strong></td>
<td>6 (12)</td>
<td>3 (11)</td>
<td>3 (14)</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Family history, n (%)</strong></td>
<td>17 (34)</td>
<td>7 (25)</td>
<td>10 (45)</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>OMI, n (%)</strong></td>
<td>20 (40)</td>
<td>10 (36)</td>
<td>10 (45)</td>
<td>0.57</td>
</tr>
<tr>
<td><strong>Peripheral artery disease, n (%)</strong></td>
<td>6 (12)</td>
<td>2 (7)</td>
<td>4 (18)</td>
<td>0.39</td>
</tr>
<tr>
<td><strong>Betablockers, n (%)</strong></td>
<td>41 (82)</td>
<td>22 (79)</td>
<td>19 (86)</td>
<td>0.71</td>
</tr>
<tr>
<td><strong>Statins, n (%)</strong></td>
<td>46 (92)</td>
<td>27 (96)</td>
<td>19 (86)</td>
<td>0.31</td>
</tr>
<tr>
<td><strong>ACE-I or ARB, n (%)</strong></td>
<td>32 (64)</td>
<td>16 (57)</td>
<td>16 (73)</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>Calcium channel blockers, n (%)</strong></td>
<td>30 (60)</td>
<td>15 (54)</td>
<td>15 (68)</td>
<td>0.39</td>
</tr>
<tr>
<td><strong>Nitrates, n (%)</strong></td>
<td>9 (18)</td>
<td>4 (14)</td>
<td>5 (23)</td>
<td>0.48</td>
</tr>
<tr>
<td><strong>Proton pomp inhibitors, n (%)</strong></td>
<td>29 (58)</td>
<td>15 (54)</td>
<td>14 (64)</td>
<td>0.57</td>
</tr>
<tr>
<td>Platelet count (10^9/µl)</td>
<td>20.2 [16.5–23.5]</td>
<td>19.9 [17.3–23.3]</td>
<td>21.0 [15.5–23.3]</td>
<td>0.78</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>65.5±18.0</td>
<td>69.7±19.3</td>
<td>60.1±14.9</td>
<td>0.06</td>
</tr>
<tr>
<td>Hemoglobin A1c (%)</td>
<td>6.2 [5.8–6.6]</td>
<td>6.2 [5.8–6.7]</td>
<td>6.2 [5.8–6.6]</td>
<td>0.77</td>
</tr>
<tr>
<td>Insulin, n (%)</td>
<td>4 (8)</td>
<td>3 (11)</td>
<td>1 (5)</td>
<td>0.62</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>45 [37–50]</td>
<td>44 [37–50]</td>
<td>46 [37–49]</td>
<td>0.78</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>90.0 [65.0–108.0]</td>
<td>90.0 [66.0–98.5]</td>
<td>93.0 [64.0–119.0]</td>
<td>0.59</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>114 [73–161]</td>
<td>131 [84–178]</td>
<td>89 [69–144]</td>
<td>0.24</td>
</tr>
<tr>
<td>Non-HDL cholesterol (mg/dl)</td>
<td>105.0 [86.0–124.0]</td>
<td>105.0 [88.0–114.5]</td>
<td>103.0 [80.0–135.0]</td>
<td>0.97</td>
</tr>
<tr>
<td>Hs Tn-T (pg/ml)</td>
<td>6.9 [3.0–10.0]</td>
<td>6.0 [3.0–10.0]</td>
<td>10.0 [3.0–13.2]</td>
<td>0.16</td>
</tr>
<tr>
<td>Hs CRP (mg/l)</td>
<td>0.60 [0.30–1.80]</td>
<td>0.50 [0.20–1.50]</td>
<td>1.20 [0.30–2.40]</td>
<td>0.26</td>
</tr>
<tr>
<td>BNP (pg/ml)</td>
<td>38.8 [16.8–70.1]</td>
<td>29.4 [15.6–63.2]</td>
<td>56.7 [38.7–121.1]</td>
<td>0.01</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>62 [58–66]</td>
<td>63 [60–67]</td>
<td>60 [49–65]</td>
<td>0.78</td>
</tr>
<tr>
<td>Coronary lesion number</td>
<td>2.0 [1.0–2.0]</td>
<td>2.0 [1.0–2.0]</td>
<td>2.0 [1.0–2.0]</td>
<td>0.99</td>
</tr>
<tr>
<td>One vessel disease, n (%)</td>
<td>17 (34)</td>
<td>10 (36)</td>
<td>7 (32)</td>
<td></td>
</tr>
<tr>
<td>Two vessel disease, n (%)</td>
<td>25 (50)</td>
<td>13 (46)</td>
<td>12 (55)</td>
<td></td>
</tr>
<tr>
<td>Three vessel disease, n (%)</td>
<td>8 (16)</td>
<td>5 (18)</td>
<td>3 (14)</td>
<td></td>
</tr>
<tr>
<td>SYNTAX score</td>
<td>12.5 [7.0–20.5]</td>
<td>11.5 [7.0–19.0]</td>
<td>13.0 [7.0–23.5]</td>
<td>0.78</td>
</tr>
<tr>
<td>RHI</td>
<td>0.53±0.20</td>
<td>0.62±0.19</td>
<td>0.42±0.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P2Y12 reaction units</td>
<td>223±88</td>
<td>160±46</td>
<td>305±55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% inhibition (%)</td>
<td>35.0 [22.0–51.5]</td>
<td>50.5 [42.0–58.0]</td>
<td>19.0 [16.0–28.0]</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

See Table 1 for abbreviations.
Supplemental Table 7. Results of logistic regression analysis for High-RPR in the long-term treatment (> 7 days) of clopidogrel

<table>
<thead>
<tr>
<th>Variable</th>
<th>Simple Regression</th>
<th>Multiple Regression with significant factors in simple analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95%CI</td>
</tr>
<tr>
<td>Age (per year)</td>
<td>1.06</td>
<td>0.99–1.13</td>
</tr>
<tr>
<td>Male (yes)</td>
<td>0.83</td>
<td>0.26–2.69</td>
</tr>
<tr>
<td>BMI (per kg/m²)</td>
<td>1.02</td>
<td>0.85–1.21</td>
</tr>
<tr>
<td>Diabetes mellitus (yes)</td>
<td>1.07</td>
<td>0.34–3.34</td>
</tr>
<tr>
<td>Hypertension (yes)</td>
<td>2.53</td>
<td>0.58–10.9</td>
</tr>
<tr>
<td>Current smoker (yes)</td>
<td>1.32</td>
<td>0.24–7.26</td>
</tr>
<tr>
<td>eGFR (per 10 ml/min/1.73 m²)</td>
<td>0.72</td>
<td>0.50–1.03</td>
</tr>
<tr>
<td>Non-HDL-cholesterol (per 10 mg/dl)</td>
<td>0.99</td>
<td>0.84–1.20</td>
</tr>
<tr>
<td>LDL-cholesterol (per 10 mg/dl)</td>
<td>1.07</td>
<td>0.87–1.31</td>
</tr>
<tr>
<td>Log₁₀ [Triglycerides/10] (per 1.0)</td>
<td>0.11</td>
<td>0.01–1.51</td>
</tr>
<tr>
<td>Log₁₀ [Hs Tn-T] (per 1.0)</td>
<td>3.91</td>
<td>0.74–20.68</td>
</tr>
<tr>
<td>Log₁₀ [BNP/10] (per 1.0)</td>
<td>4.96</td>
<td>1.22–20.26</td>
</tr>
<tr>
<td>Log₁₀ [Hs CRP/0.1] (per 1.0)</td>
<td>2.09</td>
<td>0.73–5.94</td>
</tr>
<tr>
<td>Log₁₀ [Fibrinogen] (per 0.1)</td>
<td>1.76</td>
<td>0.99–3.14</td>
</tr>
<tr>
<td>LVEF (per 5 %)</td>
<td>0.79</td>
<td>0.59–1.06</td>
</tr>
<tr>
<td>RHI (per 0.1)</td>
<td>0.51</td>
<td>0.33–0.78</td>
</tr>
</tbody>
</table>

Hosmer-Lemeshow goodness of fit chi-square was 11.16 with a p value of 0.19.
Nagelkerke R² was 0.34.
See Supplemental Tables 1 and 2 for abbreviations.
<table>
<thead>
<tr>
<th></th>
<th>Total (n=103)</th>
<th>non-High-RPR (n=50)</th>
<th>High-RPR (n=53)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cardiovascular events, n (%)</td>
<td>17 (17)</td>
<td>6 (12)</td>
<td>11 (21)</td>
<td>0.29</td>
</tr>
<tr>
<td>Cardiovascular death, n (%)</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Nonfatal myocardial infarction, n (%)</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Unstable angina, n (%)</td>
<td>2 (2)</td>
<td>2 (4)</td>
<td>0 (0)</td>
<td>0.23</td>
</tr>
<tr>
<td>Ischemic stroke, n (%)</td>
<td>2 (1)</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Hospitalization for Heart failure decompensation, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>–</td>
</tr>
<tr>
<td>Coronary revascularization, n (%)</td>
<td>11 (11)</td>
<td>3 (6)</td>
<td>8 (15)</td>
<td>0.20</td>
</tr>
</tbody>
</table>

See Supplemental Table 1 for abbreviations.

<table>
<thead>
<tr>
<th></th>
<th>Total (n=103)</th>
<th>non-High-RPR208 (n=41)</th>
<th>High-RPR208 (n=62)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cardiovascular events, n (%)</td>
<td>17 (17)</td>
<td>4 (10)</td>
<td>13 (21)</td>
<td>0.18</td>
</tr>
<tr>
<td>Cardiovascular death, n (%)</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Nonfatal myocardial infarction, n (%)</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Unstable angina, n (%)</td>
<td>2 (2)</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Ischemic stroke, n (%)</td>
<td>2 (1)</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Hospitalization for Heart failure decompensation, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>–</td>
</tr>
<tr>
<td>Coronary revascularization, n (%)</td>
<td>11 (11)</td>
<td>2 (5)</td>
<td>9 (15)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

See Supplemental Table 1 for abbreviations.
Supplemental Figure 1.

Supplemental Figure 2.
Supplemental Figure 3.

A

![Graph A](image)

B

![Graph B](image)
**Figure Legend**

**Supplemental Figure 1.**
Kaplan-Meier analysis for the probability of cardiovascular events in patients with high residual platelet reactivity (High-RPR) and non-High-RPR defined by the cut-off value of 230 PRU at 1340 days (mean: 657 days, median: 620 days) after enrollment.

**Supplemental Figure 2.**
Kaplan-Meier analysis for the probability of cardiovascular events in patients with high residual platelet reactivity (High-RPR) and non-High-RPR defined by the cut-off value of 208 PRU at 1340 days (mean: 657 days, median: 620 days) after enrollment.

**Supplemental Figure 3.**
(A) Platelet reactivity of platelet-rich plasma expressed the aggregation response over the measured time (aggregation units minute; AU min) in HCAECs treated with or without L-NAME.
(B) Platelet count of platelet-rich plasma after the incubation with HCAECs treated by L-NAME or vehicle. Bars are mean ± SEM values.