Cigarette Smoking Does Not Enhance Clopidogrel Responsiveness After Adjusting VerifyNow P2Y12 Reaction Unit for the Influence of Hemoglobin Level

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ABSTRACT

OBJECTIVES The authors performed this analysis to examine whether the enhanced clopidogrel responsiveness in current smokers is maintained after adjusting the influence of hemoglobin on VerifyNow P2Y12 reaction unit (PRU).

BACKGROUND PRU is consistently reported to be lower in current smokers. However, PRU has a significant inverse relationship with hemoglobin level, and smokers have higher hemoglobin levels. Because the association between PRU and hemoglobin is likely to be an in vitro phenomenon, we hypothesized that the observed difference in PRU between nonsmokers and current smokers is the result of confounding effect of hemoglobin rather than true difference in platelet reactivity.

METHODS Three cohorts were combined for the analysis (SNUBH [Seoul National University Bundang Hospital], n = 459; CILON-T [influence of CILostazol-based triple antiplatelet therapy ON ischemic complication after drug-eluting stentT implantation], n = 715; HOST-ASSURE [Harmonizing Optimal Strategy for Treatment of coronary artery stenosis - sAfety & effectivevenesSS of drug-elUting stents & antiplatelet REgimen], n = 1,357). The final combined cohort consisted of 1,314 patients who underwent percutaneous coronary intervention and had VerifyNow P2Y12 assay results. General linear model (analysis of covariance) was used to control the effect of hemoglobin on PRU.

RESULTS A significant inverse correlation was observed between PRU and hemoglobin ($r = -0.389; p < 0.001$). Current smokers showed a significantly higher hemoglobin level (13.5 ± 1.6 vs. 14.4 ± 1.5; $p < 0.001$) but lower PRU level (230.1 ± 90.7 vs. 212.2 ± 83.6; $p < 0.001$). After adjusting the influence of hemoglobin on PRU, there was no difference in PRU between nonsmokers and current smokers (224.1 [95% confidence interval: 218.7 to 229.5] vs. 225.3 [95% confidence interval: 217.2 to 233.1]; $p = 0.813$).

CONCLUSIONS The observed difference in PRU between nonsmokers and current smokers is largely attributable to the difference in hemoglobin level. Enhanced clopidogrel responsiveness in cigarette smokers is not confirmed in this study and the concept of the smokers’ paradox needs further validation. (J Am Coll Cardiol Intv 2016;9:1680–90) © 2016 by the American College of Cardiology Foundation.

CLOPIDOGREL has been prescribed widely to prevent major adverse cardiac events (MACE) after percutaneous coronary intervention (PCI). However, there is a significant interindividual variation in clopidogrel responsiveness (1,2) and resistance to clopidogrel is associated with an increased risk of MACE (3,4). Among several factors that influence platelet response to
clopidogrel, cigarette smoking is reported to be associated with enhanced clopidogrel responsiveness (5-7). The reason for this phenomenon is not clear, although several mechanisms have been suggested, including those regarding cytochrome P450 enzyme system.

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It is reported consistently that higher hemoglobin or hematocrit levels are associated with a lower VerifyNow (Accumetrics, San Diego, California) P2Y12 reaction unit (PRU) and vice versa (8-11). Furthermore, this association between hemoglobin (or hematocrit) and PRU is thought to be a laboratory error rather than true difference in platelet reactivity (8,9). Meanwhile, smoking is associated with increased levels of hemoglobin and hematocrit (12-14). It is not clear whether smoking is associated with a lower PRU value due to altered pharmacodynamics of clopidogrel in smoking patients or its association with increased hemoglobin level, which would lead to a lower PRU value. Gremmel et al. (15) measured platelet reactivity with light transmission aggregometry, VerifyNow P2Y12 assay, vasodilator-stimulated phosphoprotein phosphorylation assay, multiple electrode platelet aggregometry (MEA), and Impact-R at the same time in 288 patients undergoing PCI. Among 5 assays, smoking was associated with enhanced clopidogrel responsiveness only in the VerifyNow P2Y12 assay. Because most studies reporting smokers’ paradox used VerifyNow P2Y12 assay to measure platelet reactivity (6,7,16-20), the association among PRU, smoking, and hemoglobin should be elucidated. We conducted this analysis to evaluate whether the relationship between smoking and enhanced clopidogrel responsiveness is a true in vivo phenomenon that is independent of hemoglobin level or is just a laboratory error due to the confounding effect of hemoglobin.

METHODS

STUDY POPULATION. Three cohorts were used to test our hypothesis: 1) SNUBH (Seoul National University Bundang Hospital) cohort; 2) CILON-T (influence of cilostazol-based triple antiplatelet therapy on ischemic complication after drug-eluting stent implantation) cohort; and 3) HOST-ASSURE (Harmonizing Optimal Strategy for Treatment of coronary artery stenosis-safety & effectiveness of drug-eluting stents & antiplatelet REGimen) cohort. The SNUBH cohort is a retrospective cohort and CILON-T and HOST-ASSURE cohorts are originally multicenter randomized clinical trials. In the present study, patients who had VerifyNow P2Y12 assay results were included for the analysis and cilostazol users were excluded. Main results and inclusion and exclusion criteria of each cohort are published previously (8,21-23). Written informed consent was obtained for CILON-T and HOST-ASSURE cohorts. Institutional review board of Seoul National University Bundang Hospital approved the waiver of written informed consent because the SNUBH cohort is a retrospective study. The protocols of this study were consistent with the ethical guidelines of the 1975 Helsinki Declaration. The institutional review boards of each hospital ensured appropriate ethical and bioethical conduct. The protocols of CILON-T and HOST-ASSURE have been registered (NCT00776828 and NCT01267734, respectively) and detailed protocols have been published elsewhere (24,25).

INTERVENTIONAL PROCEDURES. In general, interventional procedures of the 3 cohorts were similar. Before undergoing PCI, patients were given 100 mg aspirin and 75 mg clopidogrel daily for at least 7 days. Loading doses of aspirin (300 mg) and clopidogrel (300 to 600 mg) were given to patients who had not taken them before. After PCI, aspirin and clopidogrel were administered for at least 6 months. If randomized to triple antiplatelet therapy group (CILON-T and HOST-ASSURE), patients were given an additional 200 mg of cilostazol just before the interventional procedure and then 100 mg twice daily for 6 months in CILON-T and 1 month in HOST-ASSURE. In the SNUBH cohort, cilostazol was prescribed based on the operator’s clinical decision. Patients randomized to double-dose clopidogrel dual antiplatelet therapy group in HOST-ASSURE were maintained on 150 mg/day maintenance dose of clopidogrel for 1 month. Unfractionated heparin was given during PCI with a target range of activation coagulation time of 250 to 300 ms. Glycoprotein IIb/IIIa inhibitor was used based on the operator’s decision. Coronary angiography and PCI were performed in accordance with the current standard technique.

BLOOD SAMPLING AND PLATELET FUNCTION TEST. Blood samples for baseline laboratory evaluation including complete blood cell count were collected before the initial PCI procedure. Residual platelet function after antiplatelet treatment was evaluated by the VerifyNow P2Y12 assay, and the results are reported as PRU. The device was used according to the instructions made by the manufacturer. Blood samples for platelet function test were taken from each patient after PCI and before discharge.
TABLE 1  Constitution of Each Cohort

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Study design</th>
<th>Patient number</th>
<th>Enrollment period</th>
<th>Patient characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNUBH (n = 459)</td>
<td>Single-center retrospective cohort</td>
<td>Total: 462 patients</td>
<td>From September 2006 to June 2009</td>
<td>All-comers undergoing PCI with a few exclusion criteria</td>
</tr>
<tr>
<td>Host-Assure (n = 1,357)</td>
<td>Multicenter, randomized, clinical trial</td>
<td>Total: 3,755 patients</td>
<td>From June 2010 to November 2011</td>
<td>All-comers undergoing PCI with a few exclusion criteria</td>
</tr>
<tr>
<td>CILON-T (n = 715)</td>
<td>Multicenter, randomized, clinical trial</td>
<td>Total: 915 patients</td>
<td>From September 2006 to June 2009</td>
<td>All-comers undergoing PCI with a few exclusion criteria</td>
</tr>
</tbody>
</table>

In SNUBH cohort, platelet function was also measured with MEA (Multiplate, Dynabyte, Munich, Germany), a whole blood impedance aggregometer. Adenosine diphosphate (ADP)-induced platelet aggregation in whole blood was assessed as previously described in other studies (26). In SNUBH cohort, aggregation was quantified as units ([AU·min]/10). All materials used in the cohort were obtained from the manufacturer.

DEFINITION OF SMOKING. In the CILON-T and HOST-ASSURE cohorts, smoking status was classified into current smokers, former smokers, and never smokers. Each category is defined as follows: 1) current smokers are those who smoked at least 100 cigarettes in their lifetime, and continued smoking cigarettes within 1 month of the enrollment period; 2) former smokers are those who smoked at least 100 cigarettes in their lifetime, but have not smoked within 1 month of the enrollment period; and 3) never smokers are those who smoked <100 cigarettes in their lifetime. Non-smokers were defined as never smokers plus former smokers. In the SNUBH cohort, patients were recorded as current smokers if they smoked at least 100 cigarettes and continued smoking within 1 month of the index PCI. Never smokers and former smokers were not separated and were recorded as nonsmokers.

CLINICAL OUTCOME. For the clinical outcome comparison between current smokers and nonsmokers, MACE was defined as a composite of cardiac death, myocardial infarction, and ischemic stroke. The cause of death was considered as cardiac unless there was a clear evidence of noncardiac origin. Myocardial infarction was defined as according to the published guidelines (27). Ischemic stroke was defined as a new focal neurologic deficit of vascular origin lasting more than 24 h, which was confirmed to be nonhemorrhagic by computed tomography or magnetic resonance imaging. Definite and probable stent thrombosis and bleeding events were also analyzed.

STATISTICAL ANALYSIS. Statistical analysis was performed using SPSS version 18.0 (SPSS Inc., Chicago, Illinois). Continuous variables are presented as mean ± SD and unpaired Student t test was used for the comparison. Analysis of variance with post hoc Bonferroni correction was used to compare continuous variables with more than 2 groups. Categorical variables were compared using the chi-square test or Fisher exact test as appropriate. For correlation analysis, Pearson’s correlation coefficient r was used. Clinical outcome analysis was performed through the Kaplan-Meier method and the difference between groups was compared with the log-rank test. Univariate linear regression analysis was performed to calculate the regression coefficient between hemoglobin and PRU. A general linear model (analysis of covariance) was applied to quantify the impact of cigarette smoking on clopidogrel responsiveness while controlling the effect of hemoglobin. Among several variables that were different between current smokers and nonsmokers, age and sex status were adjusted in addition to hemoglobin because these variables are reported to influence PRU value. Estimated means calculated by general linear model are presented as mean values (95% confidence intervals).

To address the potential problem of multicollinearity, multivariate linear regression analysis was performed and variance inflation factor was calculated. Variance inflation factor lower than 10 was considered as nonsignificant multicollinearity. Cohort-level meta-analysis of the 3 cohorts was performed using random effects model (DerSimonian and Laird method). Differences in estimated means and its standard errors were used for effect size calculation using generic inverse variance method. Heterogeneity was assessed with F statistic. Meta-analysis was performed with Comprehensive Meta-Analysis program (Biostat,
Englewood, NJ). All significance tests were 2-tailed, and \( p < 0.05 \) was considered significant.

RESULTS

STUDY POPULATION. Study designs, patient numbers, enrollment periods, and patient characteristics of each cohort are presented in Table 1. The SNUBH, CILON-T, and HOST-ASSURE cohorts consisted of 459, 715, and 1,357 patients who performed VerifyNow P2Y12 assay, respectively. Combining 3 cohorts resulted in a total of 2,394 patients (among 459 patients of SNUBH cohort, 137 patients were enrolled in HOST-ASSURE trial and these patients were excluded from the combined cohort). After removing 1,080 cilostazol users, the final combined cohort consisted of 1,314 patients (Figure 1).

Baseline characteristics of the final combined cohort are summarized in Table 2.

ASSOCIATION AMONG PRU, HEMOGLOBIN, AND SMOKING STATUS. In the combined cohort of 1,314 patients, PRU and hemoglobin showed a significant inverse correlation \( (r = -0.389; p < 0.001) \) (Figure 2A). Unstandardized regression coefficient calculated by univariate linear regression analysis was -21.4, which means that the PRU is decreased by 21.4 for every 1 mg/dl increase in hemoglobin level. There were significant differences in PRU across quartiles of hemoglobin \( (p < 0.001) \) (Figure 2B). Consistent with previous reports \((6,7,15,16,20,28)\), PRU was significantly lower in current smokers compared with non-smokers \((230.1 \pm 90.7 \text{ vs. } 212.2 \pm 83.6; p < 0.001)\) (Figure 2C). At the same time, current smokers showed a significantly higher hemoglobin level \((13.5 \pm 1.6 \text{ vs. } 14.4 \pm 1.5; p < 0.001; \text{Figure 2D})\) compared with non-smokers. In Kaplan-Meier survival curve analysis, there was no difference in the incidence rate of MACE between nonsmokers and current smokers \((3.2\% \text{ vs. } 1.9\%); \text{log-rank } p = 0.949)\) (Online Figure 1). Cumulative incidence of definite and probable stent thrombosis and bleeding events also did not differ between nonsmokers and current smokers (Online Table 1).

HEMOGLOBIN-ADJUSTED PRU BETWEEN NONSMokers AND CURRENT SMokers. Because PRU and hemoglobin showed a significant inverse correlation and current smokers had a significantly higher hemoglobin...
The results of separate cohorts are presented in Online Table 2. Because the CILON-T and HOST-ASSURE cohorts recorded smoking status as never, former, and current smokers, we compared raw PRU and hemoglobin-adjusted PRU of never smokers and current smokers. Although there was a significant difference in raw PRU (230.6 [95% CI: 222.6 to 238.6] vs. 207.7 [95% CI: 197.2 to 218.2]; p = 0.001) (Figure 3C) between never smokers and current smokers, there was no difference in hemoglobin-adjusted PRU (221.0 [95% CI: 213.4 to 228.7] vs. 224.1 [95% CI: 213.9 to 234.3]; p = 0.647) (Figure 3D). In baseline demographics, age, sex, prevalence of hypertension, lipid profile, initial diagnosis, and calcium channel blocker usage rate was significantly different between nonsmokers and current smokers (Table 2). Because previous study suggests that PRU is associated independently with age and sex (29), we also adjusted for age and sex in addition to hemoglobin. After adjusting the 3 variables simultaneously, there was no difference in PRU between nonsmokers and current smokers (221.5 [95% CI: 216.0 to 227.0] vs. 230.9 [95% CI: 222.6 to 239.3]; p = 0.078) (Figure 3E). Multivariate linear regression analysis also revealed that smoking status is not associated with PRU, whereas age, sex, and hemoglobin are significantly associated with PRU (Online Table 3). Variance inflation factors indicate that there is no significant multicollinearity.

In a subgroup of patients, hematocrit was also available in addition to hemoglobin (n = 981). Hematocrit also showed an inverse association with PRU (Online Table 3). Multivariate linear regression analysis also revealed that smoking status is not associated with PRU, whereas age, sex, and hemoglobin are significantly associated with PRU (Online Table 3). Variance inflation factors indicate that there is no significant multicollinearity.

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hemoglobin alone (weighted mean difference = 1.315; \( p = 0.796 \)) (Figure 4B); or 2) hemoglobin, sex, and age simultaneously (weighted mean difference = -6.245; \( p = 0.288 \)) (Figure 4C).

### MEA ADP ASSAY RESULTS BETWEEN NONSMOKERS AND CURRENT SMOKERS.

The SNUBH cohort had 362 patients who performed MEA ADP test in addition to VerifyNow P2Y12 assay (excluding cilostazol users). In contrast with PRU, there was no difference in MEA ADP assay results between nonsmokers and current smokers (26.9 [95% CI: 24.4 to 29.4] vs. 24.6 [95% CI: 22.0 to 27.3]; \( p = 0.226 \)) (Figure 5A). In addition, hemoglobin-adjusted MEA ADP was not different between nonsmokers and current smokers (27.0 [95% CI: 24.4 to 29.5] vs. 24.6 [95% CI: 21.8 to 27.3]; \( p = 0.217 \)) (Figure 5B), which suggests that the MEA ADP assay is not affected by hemoglobin.

### PRU AND HEMOGLOBIN LEVELS OF NEVER, FORMER, AND CURRENT SMOKERS.

In the CILON-T and HOST-ASSURE cohorts, nonsmokers were separated into never smokers and former smokers. PRU and hemoglobin levels of never smokers, former smokers, and current smokers are presented in Figures 6A and 6B. The observed differences in PRU and hemoglobin between each comparison groups are presented in Figure 6C. Using the regression coefficient of hemoglobin and PRU (−21.4), we calculated the estimated difference in PRU of each comparison group (Figure 6C). In general, the observed difference in PRU was in accordance with the estimated difference in PRU, which means that the observed differences in PRU among never smokers, former smokers, and current smokers can largely be explained by hemoglobin differences of each group.
Without adjustment, a significant difference in PRU was observed between nonsmokers and current smokers. After adjusting the influence of hemoglobin, there was no difference in PRU between nonsmokers and current smokers. Raw PRU was significantly different between never smokers and current smokers. Never smokers and current smokers showed no difference in hemoglobin-adjusted PRU. After adjusting the influence of age, sex and hemoglobin, there was no difference in PRU between nonsmokers and current smokers. All graphs are depicted based on the results of general linear model (analysis of covariance; SPSS version 18.0). Diamonds denote estimated mean values. Whiskers denote 95% confidence intervals. Abbreviations as in Figure 2.
DISCUSSION

In this study, we found that cigarette smoking does not improve clopidogrel responsiveness when PRU is adjusted for hemoglobin. Because PRU, hemoglobin, and smoking status are closely associated, hemoglobin should be considered as a covariate when evaluating the influence of smoking on clopidogrel responsiveness.

**FIGURE 4** Cohort-Level Meta-Analysis

<table>
<thead>
<tr>
<th>Study name</th>
<th>Difference in mean</th>
<th>Standard error</th>
<th>z-value</th>
<th>p-value</th>
<th>Relative weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHUH</td>
<td>36.070</td>
<td>4.908</td>
<td>0.016</td>
<td>0.000</td>
<td>31.23</td>
</tr>
<tr>
<td>GLOVNT</td>
<td>5.425</td>
<td>9.658</td>
<td>0.503</td>
<td>0.617</td>
<td>31.78</td>
</tr>
<tr>
<td>HOST-ASSURE</td>
<td>21.717</td>
<td>7.955</td>
<td>2.694</td>
<td>0.007</td>
<td>37.02</td>
</tr>
</tbody>
</table>

Test of overall effect (random-effects model)

Heterogeneity: $P = 0.003$

Non-smoker has lower PRU

Meta Analysis

**FIGURE 5** Comparison of MEA ADP Assay Results

Both MEA ADP (A) and hemoglobin-adjusted MEA ADP (B) did not differ between nonsmokers and current smokers. All graphs are depicted based on the results of general linear model (analysis of covariance; SPSS 18.0). **Diamonds** denote estimated mean values. **Whiskers** denote 95% CIs. MEA ADP = multiple electrode aggregometry adenosine diphosphate test; other abbreviations as in Figures 2 and 3.
responsiveness. To the best of our knowledge, this is the first study that took hemoglobin level into account before defining the relationship between smoking status and clopidogrel responsiveness.

**CURRENT CONCEPT OF THE SMOKERS’ PARADOX.** In several large scale clinical trials, nonsmokers did not benefit from clopidogrel therapy, whereas smokers showed a significant reduction in primary outcome endpoints related to clopidogrel treatment (5,16,30). This phenomenon is called the smokers’ paradox and enhanced clopidogrel responsiveness in smokers is suggested as one of the underlying mechanisms. Clopidogrel, a prodrug, needs to be converted to active metabolite by cytochrome P450 enzyme system to bind to P2Y12 ADP receptor (6,16). It is believed that the induction of cytochrome P450 1A2 and 2B6 by cigarette smoking is responsible for enhanced clopidogrel responsiveness in smokers (6,7,16). Park et al. (6) reported that enhanced clopidogrel responsiveness was only observed in cytochrome P450 1A2 A-allele carriers (85.1% of Korean population according to the study) suggesting that cytochrome P450 1A2 plays a key role in clopidogrel metabolism. A dose-response relationship between cigarette smoking and clopidogrel-related antiplatelet effect was also demonstrated (19).

**INCONSISTENT RESULTS ACROSS DIFFERENT PLATELET FUNCTION TESTS.** Although there is a substantial amount of data suggesting an enhanced clopidogrel response in smokers, there is also some evidence that is against the current theory of enhanced clopidogrel response in smokers. The relationship between smoking and enhanced clopidogrel response is not consistent across different platelet function tests. In the study performed by Gremmel et al. (15), platelet function tests other than VerifyNow P2Y12 assay failed to show enhanced clopidogrel response in smokers. Hochholzer et al. (31) analyzed ISAR (n = 2533), EXCELSIOR (Impact of Extent of Clopidogrel-Induced Platelet Inhibition During Elective Stent Implantation on Clinical Event Rate) (n = 1,996), EXCELSIOR-ACT (EXCELSIOR-Adjusted Clopidogrel Treatment) (n = 117), and PRINCIPLE-TIMI 44 (Prasugrel in Comparison to Clopidogrel for Inhibition of Platelet Activation and Aggregation-Thrombolysis In Myocardial Infarction 44) (n = 87) cohorts to evaluate the impact of smoking on clopidogrel responsiveness (31). They found that smoking had no influence on platelet reactivity either on clopidogrel loading dose or on different maintenance doses. Importantly, MEA ADP assay was used for ISAR cohort and light transmission aggregometry (Bio/Data Pap4 aggregometer, Mölab, Germany) for other cohorts (31). Additionally, there was no difference in MEA ADP assay results between current smokers and nonsmokers in the SNUBH cohort of our study. Taken collectively, it is probable that smokers’ paradox is valid only when VerifyNow P2Y12 assay is used for platelet function test.

**ROLE OF HEMOGLOBIN AS A CONFOUNDING FACTOR.** Prasugrel is a third-generation thienopyridine P2Y12 receptor inhibitor and the pharmacodynamics and metabolism of prasugrel is not influenced by smoking status (16,28). Therefore, it is inferable that there would be no difference in PRU between prasugrel-treated smokers and nonsmokers, because the induction of cytochrome P450 enzyme system by smoking is thought to be the cause of lower PRU in clopidogrel-treated smokers. However, in the TRILOGY ACS (Targeted Platelet Inhibition to Clarify the Optimal Strategy to Medically Manage Acute Coronary Syndromes) trial, smokers showed a lower
PRU value at multiple follow-up timepoints, regardless of whether they were allocated to prasugrel arm or clopidogrel arm (28). Regarding these data, it is possible that the induction of cytochrome P450 enzyme system by smoking is not the cause of the observed low PRU in current smokers taking clopidogrel.

Previous reports (8–11) indicate that there is a significant inverse relationship between PRU and hemoglobin. The reason for this association is still not clear. Our previous study reported that, because VerifyNow P2Y12 assay is a whole blood assay system, baseline hematocrit (or hemoglobin) levels might influence the ADP stimulation-related change in turbidity (8). Blood samples from patients who are responding adequately to clopidogrel do not aggregate in response to ADP stimulation, which would lead to low light transmission. This low light transmission is translated into a low PRU value. However, light transmission might also be influenced by hemoglobin level. Increased turbidity of blood sample due to higher hemoglobin would decrease light transmission of the device (which would lead to low PRU) and vice versa. Additionally, any change in turbidity due to platelet aggregation in patients with a high hemoglobin level would not affect the overall turbidity significantly, because baseline turbidity is already high and the device might think that platelets are not aggregating and hence a lower PRU value (8). Therefore, it is possible that hemoglobin concentration might have an influence on PRU value that is unrelated to intrinsic platelet reactivity. However, further investigation is needed to clarify the underlying mechanism.

The present study also confirms the inverse association between PRU and hemoglobin. It is also well-known that current smokers have higher hemoglobin level than nonsmokers. Because the association between PRU and hemoglobin is reported to be a laboratory artifact rather than a true difference in platelet reactivity (8,9), differences in the hemoglobin level between current smokers and nonsmokers should be adjusted before comparing PRU value. Our general linear model (analysis of covariance) revealed that there is no difference in PRU between nonsmokers and current smokers after adjusting hemoglobin as a covariate. The regression coefficient between PRU and hemoglobin was -21.4 and the observed difference in PRU between nonsmokers and current smokers was -17.9, which means that the difference in PRU between nonsmokers and current smokers can largely be explained by the confounding effect of hemoglobin. In the TRILOGY ACS trial, although unadjusted PRU was associated with adverse cardiac events, this association was lost after adjusting multiple clinical variables (22). However, hemoglobin was not adjusted in the study. It would be an area of future research to explore the impact of hemoglobin adjustment on the predictive value of PRU.

**STUDY LIMITATIONS.** First, this study is a retrospective analysis of previously constructed cohorts. Second, smoking status was recorded based on self-report rather than using biomarkers such as urinary cotinine levels. We cannot exclude the possibility of underreporting.

**CONCLUSIONS**

There was no difference in PRU between nonsmokers and current smokers after adjusting hemoglobin. Our data suggest that cigarette smoking does not improve clopidogrel responsiveness.

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**PERSPECTIVES**

**WHAT IS KNOWN?** PRU is consistently reported to be lower in current smokers. It is also well-known that there is an inverse association between PRU and hemoglobin. Because current smokers have a higher hemoglobin compared with nonsmokers, there is a possibility that the observed lower PRU in current smokers is due to the confounding effect of hemoglobin rather than smoking-induced enhanced clopidogrel metabolism.

**WHAT IS NEW?** In accordance with previous studies, PRU was significantly lower in current smokers. However, there was no difference in PRU between current smokers and nonsmokers after adjusting hemoglobin as a covariate.

**WHAT IS NEXT?** The impact of adjusting hemoglobin on the predictive value of PRU, such as major adverse cardiac events, should be examined. The reason for the inverse association between PRU and hemoglobin should be elucidated and corrected if possible.
REFERENCES


KEY WORDS cigarette smoking, clopidogrel, hemoglobin, platelet reactivity

APPENDIX For additional materials, please see the online version of this article.