High On-Clopidogrel Treatment Platelet Reactivity in Thai Patients with Chronic Stable Angina Scheduled for Percutaneous Coronary Intervention

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Objective: *To determine the prevalence, clinical profile, and risk factors of high on-clopidogrel treatment platelet reactivity in Thai patients with chronic stable angina scheduled for percutaneous coronary intervention.*
Material and Method: *The patients were prospectively recruited from the consecutive patients undergoing coronary angiography and planned for elective percutaneous coronary intervention (PCI). Ten ml of blood samples were cautiously drawn from the antecubital vein of the patients to determine the hemoglobin and platelet count. Platelet aggregation test was performed by light transmittance aggregometry using platelet-rich plasma. Platelets were stimulated with 5 μM adenosine diphosphate (ADP). Platelet aggregation was expressed as the maximal percent change in light transmittance from baseline. High on-clopidogrel treatment platelet reactivity was defined as post treatment maximal platelet aggregation >46% with 5 μmol/l ADP used as agonist.*
Results: *The present study consecutively enrolled two hundred four patients diagnosed with chronic stable angina planned for PCI. Seventy-nine patients demonstrated the high on-clopidogrel treatment platelet reactivity (38.7%). Among these patients, 48% were men with a mean age of 66 years. Diabetes mellitus and chronic kidney disease were detected in 34.2%. Original clopidogrel (Plavix®) was prescribed in 72% of the patients and 28% received generic clopidogrel (Apolets®). The prevalence of high on-clopidogrel treatment platelet reactivity increased in the older patients, patients with CKD and patients receiving angiotensin receptor blockers (ARB). However, from multivariate analysis, none of the risk factors, including age, BMI, diabetes mellitus, smoking, CKD, ARB use, and type of clopidogrel (Plavix® versus Apolets®) had a statistically significant association with the high on-clopidogrel treatment platelet reactivity.*
Conclusion: *The prevalence of high on-clopidogrel treatment platelet reactivity in the present study was 38.7%. No significant association was demonstrated between age, BMI, diabetes mellitus, smoking, CKD, ARB use, type of clopidogrel, and high on-clopidogrel treatment platelet reactivity.*

Keywords: *High on-clopidogrel treatment platelet reactivity, Clopidogrel resistance, Clopidogrel non-responsiveness, Clopidogrel, Platelet aggregation*

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Platelet activation and aggregation play an important role in the pathogenesis of arterial thrombosis leading to acute coronary syndromes (ACS) and thrombotic complications during percutaneous coronary intervention (PCI) including stent thrombosis[1,2]. At the present time, dual antiplatelet therapy (DAPT) with aspirin and clopidogrel is proven to be superior to aspirin alone in the patients undergoing PCI and stent implantation for reducing the incidence of ischemic events and stent thrombosis[3,4]. However, a significant number of patients still had adverse cardiovascular events including stent thrombosis after successful PCI[5-7]. These adverse events were in part believed to relate with a condition, namely clopidogrel resistance or high on-clopidogrel treatment platelet reactivity (HCTPR)[14]. The aim of the present study was to determine the prevalence, clinical profile, and possible

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risk factors of this condition in Thai patients with chronic stable angina planned for elective PCI.

Material and Method

The patients diagnosed chronic stable angina were consecutively recruited from the patients undergoing coronary angiography and planned for elective percutaneous coronary intervention (PCI) at Siriraj Hospital between March 2011 and February 2012. The diagnosis of chronic stable angina was done by using typical ischemic chest pain or one of the investigations including exercise stress test, nuclear imaging, or cardiac MRI. All patients who were ≥20 years old and who had received the combination of aspirin 81-300 mg/d and clopidogrel 75 mg/d for at least seven days were eligible for enrollment. Informed consent was obtained from every patient. The exclusion criteria included history of abnormal bleeding, taking other antiplatelet agents (ticopidine, dipyridamole, cilostazol and NSAIDs), history of acute MI within 48 hours, stroke within 3 months, elicited drug or alcohol abuse, serum creatinine >2.0 mg/dl, platelet count <100,000/mm^3, and hematocrit (Hct) <30%.

This research was approved by Siriraj Ethical Approval No 249/2552 (EC2).

Blood sample and assay procedure

10 ml of blood samples were cautiously drawn from the antecubital vein of the patients. The blood then was collected in 3.2% sodium citrate tube for platelet aggregation test and EDTA tube for analysis of hemoglobin and platelet count. The hemoglobin and platelet count were analyzed by automated Hematology Analyzer (XT 200i Advia 120).

Platelet aggregation test was performed by light transmittance aggregometry using platelet-rich plasma and platelet count was adjusted to between $200x10^3$ and $300x10^3/mm^3$. Platelets were stimulated with 5 μM adenosine diphosphate (ADP). Aggregation was performed with a Lumiaggregation Module Series 1008. Platelet aggregation was expressed as the maximal percent change in light transmittance from baseline. The extent of aggregation was defined as the maximal light transmission at 6 min after addition of the agonist, with platelet-poor plasma used as a reference. Intra-assay coefficient of variation of the test was less than 10%. The technicians who performed the platelet aggregation test did not know about specific information of the study population individuals.

High on-clopidogrel treatment platelet reactivity (HCTPR) was defined as post treatment maximal platelet aggregation >46% with 5 μmol/l ADP used as agonist[8,14].

Statistical analysis

The subject characteristics were described using descriptive statistics, including means, standard deviation, and percentage. The normality of distribution of the variables was examined with the Kolmogorov-Smirnov. The association of normally distributed variables was determined with use of the Student's t-test to compare mean and Chi-square test to compare proportion between two groups. For non-normally distributed variables, the Mann-Whiney U test or Fisher exact test was used, in cases of continuous or dichotomous variables, respectively. The variables found to be significantly associated with the high on-clopidogrel treatment platelet reactivity from the previous studies and in univariate analysis were entered in a multivariable enter (conditional), multiple logistic regression model. For all tests performed, a two-tailed p-value <0.05 was considered as denoting statistical significance. The statistical software SPSS, version 18.0 was employed for all the analyses performed

Results

The present study enrolled two hundred four patients diagnosed with chronic stable angina planned for PCI. Among these patients, 60.8% were men. The mean age of the study population was 64 years. The body mass index (BMI) was 25.8 and 25.1 kg/m^2 in male and female, respectively. Diabetes mellitus was detected in 38.7% of the patients and 26% of the study patients had chronic kidney disease (CKD) (as defined by GFR <60 ml/min). Two hundred one patients (98.5%) had taken 75 mg/d of clopidogrel. The duration of clopidogrel treatment before platelet testing and the other baseline characteristics between the two groups are shown in Table 1. The distribution of platelet aggregation stimulated with 5 μM adenosine diphosphate (ADP) is demonstrated in Fig. 1 and 2. The mean platelet aggregation in response to 5 μM adenosine diphosphate (ADP) in patients with and without high on-clopidogrel treatment platelet reactivity were 59.8±9.5% and 27.8±12%; (p = 0.01), respectively.

In the present study, original clopidogrel (Plavix®) was prescribed in 72% (n = 147) of the patients and 28% (n = 57) received generic clopidogrel (Apolets®). The prevalence of high on-clopidogrel treatment platelet reactivity in all study patients

Table 1. Baseline characteristics of the study patients (n = 204)

	High on-clopidogrel platelet reactivity (n = 79)	Normal clopidogrel response (n = 125)	p-value
Age (yr)	66.0±10.8	63.0±12.7	0.04
Male sex	44 (48.0)	80 (76.0)	0.24
Body mass index (kg/m^2)	25.8±4.4	25.1±4.4	0.27
Smoking	7 (8.9)	21 (16.8)	0.11
Diabetes mellitus	27 (34.2)	52 (41.6)	0.29
Hypertension	62 (78.5)	94 (75.2)	0.59
Hypercholesterolemia	61 (77.2)	82 (65.6)	0.08
Prior MI	28 (35.4)	42 (33.6)	0.79
Prior PCI	29 (36.7)	51.125 (40.8)	0.56
Prior CABG	6 (7.6)	6 (4.8)	0.54
Prior stroke	3 (3.8)	5 (4.0)	1.00
CHF	12 (15.2)	21 (16.8)	0.76
PPI use	39 (49.4)	50 (40.0)	0.19
CKD*	27 (34.2)	26 (20.8)	0.03
Creatinine clearance (mL/min)	57.2±27.9	63.9±29.7	0.11
Chronic liver disease	0	1 (0.8)	1.00
Duration of clopidogrel use before platelet function testing			0.21
<2 weeks	11 (13.9)	17 (13.6)	
2-4 weeks	13 (6.5)	17 (13.6)	
4-8 weeks	7 (8.9)	19 (15.2)	
>8 weeks	48 (60.8)	72 (57.6)	
Medication			
Beta blocker	64 (81.0)	92 (73.6)	0.22
ACEI	32 (40.5)	57 (45.6)	0.48
ARB	24 (30.4)	21 (16.8)	0.02
Statin	68 (86.1)	112 (89.6)	0.45

Data are expressed as number (%) and mean ± standard deviation.
* CKD was defined as creatinine clearance <60 mL/min.
ACEI = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; CABG = coronary artery bypass graft; CHF = congestive heart failure; CKD = chronic kidney disease; MI = myocardial infarction; PCI = percutaneous coronary intervention; PPI = proton pump inhibitor

(n = 204) was 38.7%. This prevalence increased significantly in the older patients, patients with CKD and patients receiving angiotensin receptor blockers (ARB).

When type of clopidogrel was taken into account, it was found that 36.1% of patients receiving Plavix® and 45.6% of those receiving Apolets® had high on-clopidogrel treatment platelet reactivity respectively. However, by univariate analysis, this difference did not show statistical significance.

The multivariate analysis to determine the risk factors for the presence of HCTPR in these patients was performed as shown in Table 2. The variables entered into the model included age, BMI, diabetes mellitus, smoking, CKD, ARB use, and Apolets®. It was found that no risk factor had a statistically significant association with the high on-clopidogrel treatment platelet reactivity.

Discussion

From the present study, it was demonstrated that 38.7% of the patients with chronic stable angina had high on-clopidogrel treatment platelet reactivity or clopidogrel non-responsiveness based on platelet function test by light transmittance aggregometry. This prevalence was higher compared with the previous

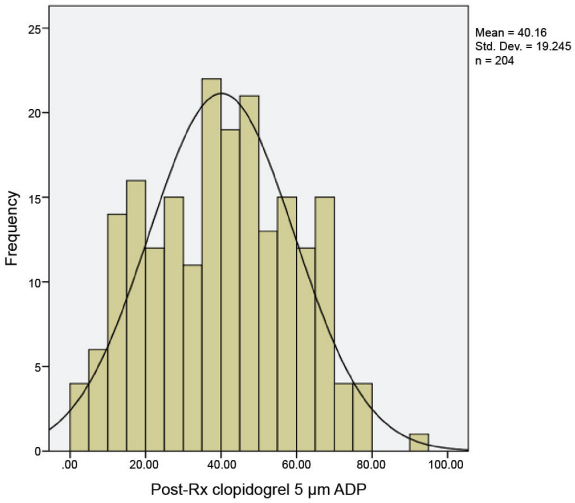

Fig. 1 Distribution of platelet aggregation in response to 5 µM adenosine diphosphate (ADP) stimulation after receiving clopidogrel therapy.

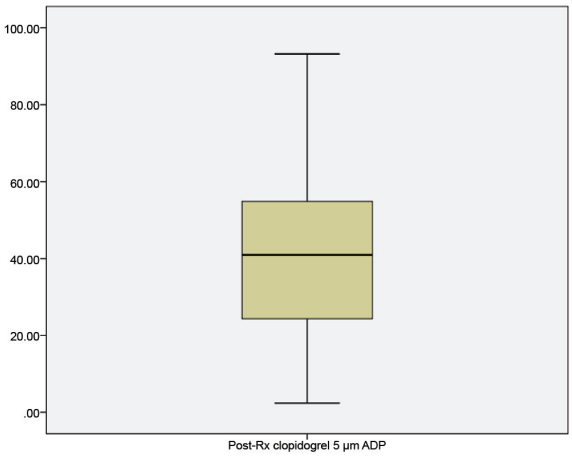

Fig. 2 Median and interquartile range of platelet aggregation in response to 5 µM adenosine diphosphate (ADP) stimulation after receiving clopidogrel therapy.

studies reporting the prevalence of clopidogrel non-responsiveness of 4 to 30%[9-13].

There are many reasons to explain this difference. First, the definition of clopidogrel non-responsiveness widely differed between the studies. In the present study, the authors used the definition that could predict the ischemic event in the patients who underwent PCI with stenting[14]. The sensitivity and specificity of this definition were 63% and 82% respectively[8]. Second, there are different techniques used to measure the extent of platelet aggregation including light transmittance aggregometry (LTA), Verify Now P2Y12 assay, Multiplate analyzer, PFA-100, Plateletworks, Thromboelastagraph platelet mapping system, and vasodilator-stimulated phosphoprotein (VASP)[14]. However, LTA, used in the present study, is still considered as the gold standard to evaluate the extent of platelet aggregation even though it takes some limitations[15]. Finally, the presence of some contributing factors to greater baseline platelet reactivity in each of the studies, such as acute coronary syndrome (ACS), increased body mass index (BMI) ≥25 kg/m^2, diabetes mellitus, poor metabolizers (2 loss-of-function CYP2C19 alleles), and poor compliance, were not similar.

From univariate analysis of the present study, it showed that increased age, CKD and ARB use are significant different between the study groups. However, the multivariate analysis as shown in Table 2 could not demonstrate that age, BMI, diabetes mellitus, smoking, CKD, and type of clopidogrel (Plavix® versus Apolets®) were the significant risk factors to high on-clopidogrel treatment platelet reactivity or clopidogrel non-responsiveness.

Table 2. Multivariate analysis of risk factors for high on-clopidogrel treatment platelet aggregation

Factor	n	High on-clopidogrel treatment, n (%)	Crude OR (95% CI)	Adjusted OR (95% CI)	p-value
Age	204	79/204 (38.7)	1.03 (1.00-1.05)	1.02 (0.99-1.05)	0.15
BMI	204	79/204 (38.7)	1.04 (0.97-1.11)	1.04 (0.97-1.12)	0.29
DM	79	27/79 (34.2)	0.73 (0.41-1.31)	0.60 (0.32-1.13)	0.11
Smoking	28	7/28 (25.0)	0.48 (0.19-1.19)	0.60 (0.23-1.55)	0.29
CKD	53	27/53 (51.0)	1.98 (1.05-3.73)	1.96 (0.97-3.97)	0.06
Apolets®	57	26/57 (45.6)	1.49 (0.88-2.77)	1.38 (0.72-2.64)	0.33
ARB	45	24/45 (53.0)	2.16 (1.11-4.23)	1.94 (0.95-3.97)	0.07

Apolets® = generic clopidogrel; ARB = angiotensin receptor blocker; BMI = body mass index; CKD = chronic kidney disease; DM = diabetes mellitus

The authors study results are not similar to the previous studies recognizing that increased BMI and diabetes mellitus were associated with high on-clopidogrel treatment platelet reactivity[12,16,17]. This may partly be related to the small number of diabetic patients in our study and the different method used for assessing the platelet aggregation tests.

Conclusion

The present study has demonstrated the existence of high on-clopidogrel treatment platelet reactivity in Thai patients with chronic stable angina. The prevalence of this condition is 38.7%. No significant association between age, BMI, diabetes mellitus, CKD, type of clopidogrel (Plavix® versus Apolets®) and high on-clopidogrel treatment platelet reactivity.

Study limitations

There are some limitations to the present study. Clopidogrel use was assessed by discussion with the patient without confirmation by pill counts. Platelet aggregation studies were performed only at a baseline with different prior duration of clopidogrel treatment, therefore, different variable response to clopidogrel may exist, even though the present study did not show significant difference for the prior duration between the two groups. Laboratory variability in the isolation of platelet rich plasma may influence the measurement of platelet aggregation. Finally, the study population may be not large enough to demonstrate the significant risk factors to high on-clopidogrel treatment platelet reactivity or clopidogrel non-responsiveness.

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Potential conflicts of interest

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References

1. Selwyn AP. Prothrombotic and antithrombotic pathways in acute coronary syndromes. Am J Cardiol 2003; 91: 3H-11H.
2. Mak KH, Belli G, Ellis SG, Moliterno DJ. Subacute stent thrombosis: evolving issues and current concepts. J Am Coll Cardiol 1996; 27: 494-503.
3. Steinhubl SR, Berger PB, Mann JT III, Fry ET, DeLago A, Wilmer C, et al. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. JAMA 2002; 288: 2411-20.
4. Mehta SR, Yusuf S, Peters RJ, Bertrand ME, Lewis BS, Natarajan MK, et al. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. Lancet 2001; 358: 527-33.
5. Reynolds MR, Rinaldi MJ, Pinto DS, Cohen DJ. Current clinical characteristics and economic impact of subacute stent thrombosis. J Invasive Cardiol 2002; 14: 364-8.
6. Regar E, Lemos PA, Saia F, Degertekin M, Tanabe K, Lee CH, et al. Incidence of thrombotic stent occlusion during the first three months after sirolimus-eluting stent implantation in 500 consecutive patients. Am J Cardiol 2004; 93: 1271-5.
7. Cutlip DE, Baim DS, Ho KK, Popma JJ, Lansky AJ, Cohen DJ, et al. Stent thrombosis in the modern era: a pooled analysis of multicenter coronary stent clinical trials. Circulation 2001; 103: 1967-71.
8. Gurbel PA, Antonino MJ, Bliden KP, Dichiara J, Suarez TA, Singla A, et al. Platelet reactivity to adenosine diphosphate and long-term ischemic event occurrence following percutaneous coronary intervention: a potential antiplatelet therapeutic target. Platelets 2008; 19: 595-604.
9. Jaremo P, Lindahl TL, Fransson SG, Richter A. Individual variations of platelet inhibition after loading doses of clopidogrel. J Intern Med 2002; 252: 233-8.
10. Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. Circulation 2003; 107: 2908-13.
11. Mobley JE, Bresee SJ, Wortham DC, Craft RM, Snider CC, Carroll RC. Frequency of nonresponse antiplatelet activity of clopidogrel during pre-treatment for cardiac catheterization. Am J Cardiol 2004; 93: 456-8.
12. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Barrera RC, Sabate M, Fernandez C, et al. Platelet aggregation according to body mass index in patients undergoing coronary stenting: should clopidogrel loading-dose be weight adjusted? J Invasive Cardiol 2004; 16: 169-74.

13. Serebruany VL, Steinhubl SR, Berger PB, Malinin AI, Bhatt DL, Topol EJ. Variability in platelet responsiveness to clopidogrel among 544 individuals. J Am Coll Cardiol 2005; 45: 246-51.
14. Bonello L, Tantry US, Marcucci R, Blindt R, Angiolillo DJ, Becker R, et al. Consensus and future directions on the definition of high on-treatment platelet reactivity to adenosine diphosphate. J Am Coll Cardiol 2010; 56: 919-33.
15. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Alfonso F, Macaya C, Bass TA, et al. Variability in individual responsiveness to clopidogrel: clinical implications, management, and future perspectives. J Am Coll Cardiol 2007; 49: 1505-16.
16. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramirez C, Sabate M, Jimenez-Quevedo P, et al. Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. Diabetes 2005; 54: 2430-5.
17. Angiolillo DJ, Bernardo E, Ramirez C, Costa MA, Sabate M, Jimenez-Quevedo P, et al. Insulin therapy is associated with platelet dysfunction in patients with type 2 diabetes mellitus on dual oral antiplatelet treatment. J Am Coll Cardiol 2006; 48: 298-304.

ภาวะดื้อต่อการออกฤทธิ์ของ clopidogrel ในผู้ป่วยหลอดเลือดหัวใจตีบเรื้อรังที่ได้รับการรักษาโดยการขยายหลอดเลือดหัวใจ

เรวัตร พันธุ์กิ่งทองคำ, ประดิษฐ์ ปัญจวีณิน, ยิ่งยง ชินธรรมมิตร์, ดำรัส ตรีสุโกศล, ชุณหเกษม โชตินัยวัตรกุล, วิวรรณ ทังสุบุตร, ณัฐวุฒิ วงษ์ประภารัตน์, บุษกร กิจรัตนา, ปิยฉัตร ลีวานันท์

วัตถุประสงค์: *เพื่อศึกษาความชุก ลักษณะทางคลินิก รวมทั้งปัจจัยเสี่ยงของภาวะดื้อต่อการออกฤทธิ์ของ clopidogrel ในผู้ป่วยหลอดเลือดหัวใจตีบเรื้อรังที่ได้รับการขยายหลอดเลือดหัวใจ*

วัสดุและวิธีการ: *การศึกษานี้คัดเลือกผู้ป่วยที่ได้รับการวินิจฉัยว่าเป็นโรคหลอดเลือดหัวใจตีบชนิดเรื้อรัง และได้รับการฉีดสีร่วมกับการขยายหลอดเลือดหัวใจที่โรงพยาบาลศิริราชในช่วงเดือนมีนาคม พ.ศ. 2554 ถึงเดือนกุมภาพันธ์ พ.ศ. 2555 ผู้ป่วยที่เข้าการศึกษาจะได้รับยา clopidogrel อย่างน้อยเป็นเวลา 7 วัน ก่อนจะทำการตรวจการทำงานของเกล็ดเลือด วิธีตรวจการทำงานของเกล็ดเลือดจะใช้หลักการ light transmittance aggregometry และใช้สาร adenosine diphosphate (ADP) ในขนาด 5 ไมโครโมล เป็นตัวกระตุ้น ในการวินิจฉัยภาวะดื้อต่อการออกฤทธิ์ของ clopidogrel จะถือเอาค่าสูงสุดของการเกาะกลุ่มของเกล็ดเลือดมากกว่าร้อยละ 46*

ผลการศึกษา: *ผู้ป่วยที่เข้าเกณฑ์การศึกษามีจำนวน 204 ราย การตรวจการทำงานของเกล็ดเลือดพบว่ามีภาวะดื้อต่อการออกฤทธิ์ของ clopidogrel จำนวน 79 ราย (ร้อยละ 38.7) โดยผู้ป่วยมีอายุเฉลี่ย 66 ปีและเป็นเพศชายร้อยละ 48 เป็นโรคเบาหวานร้อยละ 34.2 และร้อยละ 34.2 มีการทำงานของไตทำงานผิดปกติ ยา Plavix® (original clopidogrel) และ Apolets® (generic clopidogrel) ถูกใช้ในผู้ป่วยร้อยละ 72 และร้อยละ 28 ตามลำดับ ความชุกของภาวะดื้อต่อการออกฤทธิ์ของ clopidogrel พบเพิ่มขึ้นในผู้ป่วยที่อายุมาก การทำงานของไตผิดปกติ และผู้ป่วยที่ใช้ยากลุ่ม ARB อย่างไรก็ตามจาก Multivariate analysis ไม่พบความสัมพันธ์ที่มีนัยสำคัญระหว่าง อายุ, ค่าดัชนีมวลกาย, โรคเบาหวาน, การสูบบุหรี่, การใช้ยากลุ่ม ARB, การใช้ยา Apolets® (generic clopidogrel) และภาวะดื้อต่อการออกฤทธิ์ของ clopidogrel*

สรุป: *จากการศึกษานี้พบว่าความชุกของภาวะดื้อต่อการออกฤทธิ์ของ clopidogrel ในผู้ป่วยหลอดเลือดหัวใจตีบเรื้อรังมีจำนวนร้อยละ 38.7 และไม่พบว่า อายุ, ค่าดัชนีมวลกาย, โรคเบาหวาน, การสูบบุหรี่, การใช้ยากลุ่ม ARB รวมทั้งการใช้ยา Apolets® (generic clopidogrel) มีความสัมพันธ์กับภาวะดื้อต่อการออกฤทธิ์ของ clopidogrel อย่างมีนัยสำคัญ*