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# Effects of individualized antiplatelet therapy based on *CYP2C19* genotype and platelet function on the prognosis of patients after PCI

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**Abstract. – OBJECTIVE: To evaluate the effect of individualized antiplatelet therapy based on *CYP2C19* genotype and platelet function on the prognosis of patients after percutaneous coronary intervention (PCI) compared with conventional antiplatelet therapy.**

**PATIENTS AND METHODS: Patients diagnosed with acute coronary syndromes (ACS) in Shandong Provincial Qianfoshan Hospital from December 2014 to December 2017 were included in this prospective study and randomly divided into conventional (CA) and individualized antiplatelet therapy group (IA) at 1:1 ratio. Patients in the CA group received clopidogrel 75 mg once a day (QD). Group IA was divided into extensive, intermediate, and poor metabolizers according to the results of the *CYP2C19* gene test. Three genotypes were given clopidogrel 75 mg QD, 75 mg twice daily (BID) and ticagrelor 90 mg BID respectively. After taking these medicines for a period of time, platelet function was monitored by thromboelastography (TEG) and $MA_{ADP}$ values were recorded. $MA_{ADP}$ indicates the adenosine diphosphate (ADP) induced platelet function that not inhibited by medicine. High platelet reactivity (HPR) was defined as $MA_{ADP}$ > 47mm, indicating a high risk of thrombus, and $MA_{ADP}$ ≤ 31 mm indicates a high risk of hemorrhage. For extensive metabolizers (EMs) and intermediate metabolizers (IMs) patients with HPR, the antiplatelet therapy would be changed by the clinician according to the patient's conditions. Major adverse cardiovascular events (MACE) and hemorrhage events were monitored during 1-year follow-up.**

**RESULTS: The patients with $MA_{ADP}$ > 47 mm were 89 (28.6%) in the IA group. There were 50 EMs patients with $MA_{ADP}$ > 47 mm (33.3%). Of which, there were 2 cases which changed the dosage of clopidogrel to 75 mg BID, 14 cases who changed clopidogrel to ticagrelor. There were 36 IMs patients with $MA_{ADP}$ > 47 mm (30.8%). Of which, there were 19 cases who changed clopidogrel to ticagrelor. There was no significant difference in the value of $MA_{ADP}$ between EMs and IMs patients. Within 1 year after PCI, the occurrence of MACE in the IA group was significantly lower than that in the CA group ($p$=0.010).**

**CONCLUSIONS: (1) Patients with a *CYP2C19* loss-of-function (LOF) gene who take double doses of clopidogrel overcome the decreased efficacy of clopidogrel which partly due to *CYP2C19* LOF gene, without increasing the risk of hemorrhage. (2) Individualized antiplatelet therapy based on *CYP2C19* genotype and platelet function can significantly reduce the occurrence of MACE (mainly acute non-fatal myocardial infarction) after PCI without increasing the risk of moderate or severe hemorrhage.**

*Key Words:*
Antiplatelet therapy, Clopidogrel, *CYP2C19*, Gene polymorphism, Platelet reactivity, Percutaneous coronary intervention.

**Clinical Trial Registration Number**
chictr-inc-17011550.

## Introduction

Percutaneous coronary intervention (PCI) is an important treatment for patients with acute coronary syndrome. However, stent thrombosis, myocardial infarction and other adverse cardiovascular events still occurred on patients after coronary stent implantation. Platelet activation and aggregation is one of the main mechanisms of ischemic event recurrence after PCI[1,2]. Dual antiplatelet therapy (DAPT) with aspirin in com-

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bination with a $P2Y_{12}$ receptor inhibitor (clopidogrel or ticagrelor) is one of the standard therapies for reducing ischemic events in patients with coronary heart disease after PCI[3-8].

The antiplatelet effects of clopidogrel vary widely among individuals and RACES. Many patients receiving regular doses of clopidogrel showed high on-treatment platelet reactivity (HPR)[9]. HPR is an independent risk factor for ischemic events after PCI[9,10].

Clopidogrel is an inactive prodrug. After oral administration, it is absorbed in the intestine and mediated by different cytochrome P450 enzymes in the liver. After two-step enzyme activation and metabolism, it is transformed into active substances. Then, it can inhibit the adenosine diphosphate (ADP) mediated platelet activation and aggregation by irreversibly binding to $P2Y_{12}$ receptor coupled with ADP on the platelet surface[11]. The gene polymorphism of *CYP2C19*, which is involved in the activation and metabolism of clopidogrel, is related to the individualized difference in the efficacy of clopidogrel. Ticagrelor, a new $P2Y_{12}$ receptor inhibitor, is not a precursor drug. It has a strong anti platelet aggregation effect and is not affected by the enzyme gene polymorphism in the liver. However, due to higher hemorrhage risks, side effects such as dyspnea, and social and economic factors, the number of patients using clopidogrel is still very large[12].

The first step of the activation of clopidogrel in the liver produces the intermediate metabolite 2-oxo-clopidogrel, which is mainly mediated by *CYP2C19*, *CYP1A2* and *CYP2B6*, and their contribution rates are 19.4%, 35.8% and 44.9%, respectively. The second step involves the participation of *CYP2C19*, *CYP2B6*, *CYP2C9*, *CYP3A4* and other enzymes, with the contribution rate of 20.6%, 32.9%, 6.76% and 39.8% respectively[11,13]. *CYP2C19* is the only *CYP450* enzyme that plays an important role in the two-step activation and metabolism of clopidogrel.

The gene encoding *CYP2C19* is highly polymorphic[14,15]. *CYP2C19* loss of function (LOF) allele was significantly correlated with HPR. In patients with *CYP2C19* LOF allele, the activation and metabolism rate of clopidogrel decreased, and the transformation of active substances was limited, which led to the decrease of the ability of clopidogrel to inhibit platelet aggregation *in vivo*. Among patients receiving clopidogrel, the *CYP2C19* LOF allele carriers had significantly higher rates of HPR and ischemic events than non-carriers[14,15]. The most common *CYP2C19* LOF allele is * 2 (c.681g > A; rs4244285). Its gene frequency is about 12%-15% in Caucasians and Africans, and 29% - 35% in Asians. The less common *CYP2C19* LOF * 3 (rs4986893, c.636G>A;) allele frequency in Asians is 2-9%[15,16]. This means that 25-30% of people of European and African descendant and around 50% of Asians carry at least one *CYP2C19* LOF allele. This significantly affects their ability to metabolize drugs through the ENZYME *CYP2C19*. This significantly affects their ability to metabolize drugs through *CYP2C19*.

Therefore, to explore how to choose $P2Y_{12}$ receptor inhibitors to carry out individualized antiplatelet therapy for *CYP2C19* LOF allele carriers, so that all patients can get sufficient and safe antiplatelet therapy as far as possible, and achieve the purpose of effectively preventing ischemic events without increasing the risk of hemorrhage, is a very meaningful work, and more and more people pay attention to it. However, there is still room for further research and Discussion on which individualized treatment is safer and more effective.

It was found that for patients carrying one *CYP2C19 * 2*, increasing the dose of clopidogrel could enhance the platelet inhibition system and overcome the variation of antiplatelet reactivity in these patients, but for patients carrying two *CYP2C19 * 2*, increasing the dose of clopidogrel has no evident effect[17,18]. However, the results of other studies found that doubling the dose of clopidogrel could not overcome the variation of antiplatelet reactivity of patients with one *CYP2C19*2*[19,20].

This study evaluated the efficacy and safety of individualized antiplatelet therapy based on *CYP2C19* genotype and platelet function to explore safe and effective individualized antiplatelet therapy for patients after PCI.

## Patients and Methods

### Patients

This study is a prospective study. Acute coronary syndromes (ACS) patients who received PCI in Shandong Provincial Qianfoshan Hospital from December 2014 to December 2017 were included according to inclusion and exclusion criteria. Inclusion criteria: (1) patients diagnosed with ACS (including unstable angina, acute non-ST-segment elevation myocardial infarction, and acute ST-segment elevation myocardial infarction) according to guidelines[21,22] and prepared

for PCI; (2) agree to participate in the study and sign the informed consent. Exclusion criteria: (1) no coronary stent implantation; (2) people who cannot take aspirin, clopidogrel or ticagrelor as planned for any reason; (3) serious liver and kidney function damage (glutamate transaminase more than 4 times normal, blood creatinine ≥200 μmol/L); (4) platelet count >$450\times10^9$ / L or <$100\times10^9$ / L; (5) Other anticoagulant or antithrombotic drugs were applied within 24 hours of thromboelastograph (TEG) test.

This study was approved by the Ethics Committee of Shangdong Provincial Qianfoshan Hospital. The specific operation and significance of the trial study, as well as the examination items that need to be matched, are informed to all subjects at the time of selection. The enrolled subjects have signed informed consent before participating in the study.

### Antiplatelet Therapy

All patients were randomized to conventional antiplatelet therapy (CA) and individualized antiplatelet therapy (IA) at 1:1 ratio. Patients in the IA group were immediately tested for *CYP2C19* alleles, which were classified into the following metabolic types: extensive metabolizers (EMs, no LOF allele), intermediate metabolizers (IMs, carrying one LOF allele), and poor metabolizers (PMs, carrying two LOF alleles). All patients took aspirin 100 mg once a day (QD) after enrolling. Patients in CA group were treated with clopidogrel 75 mg QD. Patients with EMs, IMs and PMs were treated with clopidogrel 75 mg QD, clopidogrel 75 mg twice a day (BID) and ticagrelor 90 mg BID respectively. Patients who did not continuously take the routine maintenance dose of the above drugs (aspirin 100 mg QD, clopidogrel 75 mg QD, ticagrelor 90 mg BID) for at least three days before the administration of the above drugs were given the loading dose of corresponding drug: Aspirin 300 mg, clopidogrel 300 mg or ticagrelor 180 mg. At least 6 hours after taking 300 mg loading dose of clopidogrel, at least 2 days after taking maintenance dose clopidogrel, at least 2 hours after taking 180 mg loading dose of ticagrelor, and at least 6 hours after taking maintenance of ticagrelor, platelet function was monitored by TEG and $MA_{ADP}$ values was recorded. $MA_{ADP}$ is the platelet-fibrin blood clot strength induced by ADP, which can respond to the ADP induced platelet function that not inhibited by drugs. HPR was defined as $MA_{ADP}$ > 47 mm, indicating a high risk of thrombus, and $MA_{ADP} \leq 31$ mm indicates a high risk of hemorrhage. For EMs patients with HPR, the antiplatelet treatment was changed or unchanged by the clinicians according to the patient's conditions: changed the dosage of clopidogrel to 75 mg BID, or changed clopidogrel to ticagrelor (90 mg BID), or maintained the original treatment. For IMs patients with HPR, the antiplatelet treatment was changed or unchanged by the clinicians according to the patient's conditions: changed clopidogrel to ticagrelor (90 mg BID), or maintained the original treatment. The treatment regimen of other patients remained unchanged. All patients continued to take dual antiplatelet drugs for at least 12 months, (Figure 1).

The interventional therapy and other drug treatment of the patients in this study are decided by clinicians according to the current guidelines[7,23,24] and clinical conditions.

At month 1, 6, and 12, outpatient or telephone follow-up was conducted, and the readmission patient medical records and examination data were reviewed to monitor medication status, efficacy end points, and safety end points.

### Clinical End Point

The primary efficacy endpoint was a composite endpoint that included nonfatal myocardial infarction[6,22,25], definite stent thrombosis[26], and all-cause death. The primary safety endpoint was moderate or severe hemorrhage, and the secondary safety endpoint was mild hemorrhage. Hemorrhage events were classified according to previous literature[27] (Type 1 was mild hemorrhage, type 2, 3, 5 was moderate or severe hemorrhage).

### CYP2C19 LOF Allele Detection

The presence of *CYP2C19* LOF allele *CYP2C19*2* (681G>A, rs4244285) and *CYP2C19**3 (636G>A, rs4986893) was detected by gene chip immediately after the patients were enrolled in the IA group. Take the whole blood samples of patients for DNA extraction. *CYP2C19 * 2* and *CYP2C19 * 3* gene regions were amplified by real-time polymerase chain reaction (PCR). Then, the *CYP2C19* allele was hybridized and analyzed. The above procedures used the kit from Sinochips Bioscience Co., Ltd. (Zhuhai, Guangdong, China) in accordance with the manufacturer's guidelines.

### Platelet Function Test

After taking clopidogrel or ticagrelor for at least 5 days, 4 ml whole blood of the patients enrolled in this study was collected, inhaled into

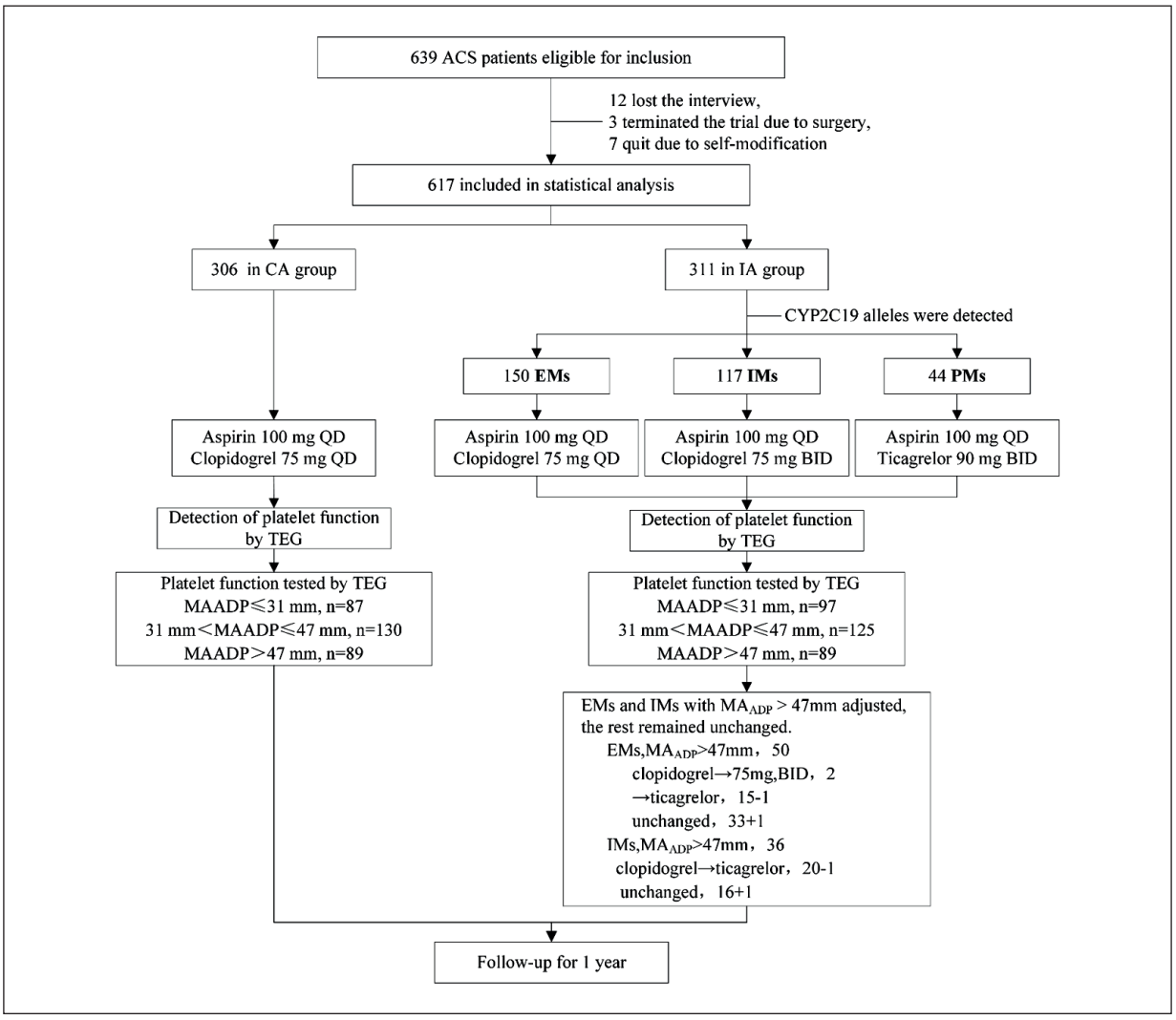


**Figure 1.** Study profile. CA, conventional antiplatelet therapy group; IA, individualized antiplatelet therapy group; EMs, extensive metabolizers; IMs, intermediate metabolizers; PMs, poor metabolizers; QD, once a day; BID, twice a day.

citrate and heparin anticoagulant blood vessels respectively, and sent to the blood laboratory of Qianfoshan Hospital in Shandong Province. Platelet function was measured by using thromboelastometer (TEG 5000, Haemoscope company of the United States) according to the manufacturer's instructions by trained technicians. All reagents are provided by Haemoscope company, including kaolin, activator F and ADP. The value of $MA_{ADP}$ which represents platelet function that can be induced by ADP while has not been inhibited by drugs, was recorded. When $P2Y_{12}$ receptor inhibitor was applied, $MA_{ADP}$ values between 31 and 47 mm suggested that platelet inhibition may be safe and effective; $MA_{ADP}$ >47 mm was defined as HPR, suggesting that the residual platelet function that was not inhibited by drugs was still strong and the risk of thrombosis was higher. $MA_{ADP}$ ≤31 mm suggested that platelet function was too low and hemorrhage risk was higher[28].

### Statistical Analysis

SPSS 19.0 software (IBM Corp., Armonk, NY, USA) was used in this study for statistical analysis. Measurement data consistent with normal distribution were expressed as mean ± standard deviation, and comparison between groups was conducted using independent sample *t*-test or one-way analysis of variance (ANOVA). Measurement data that did not conform to normal dis-

**Table I.** Demographic and clinical characteristics of patients in the CA and IA groups.

| Characteristics | Total N = 617 | CA N = 306 | IA N = 311 | *p* |
|---|---|---|---|---|
| Age, yr, $\bar{x} \pm s$ | 64.1 ± 10.6 | 64.6 ± 10.7 | 63.6 ± 10.7 | 0.893 |
| Males, n (%) | 434 (70.3%) | 214 (69.9%) | 220 (70.7%) | 0.827 |
| Risk factors | | | | |
| Smoking, n (%) | 314 (50.9%) | 152 (49.7%) | 162 (52.1%) | 0.548 |
| Drinking, n (%) | 259 (42.0%) | 131 (42.8%) | 128 (41.2%) | 0.677 |
| Hypertension, n (%) | 514 (83.3%) | 260 (85.0%) | 254 (81.7%) | 0.272 |
| Diabetes mellitus, n (%) | 261 (42.3%) | 133 (43.5%) | 128 (41.2%) | 0.562 |
| Diagnosis at admission | | | | 0.961 |
| UA, n (%) | 434 (70.3%) | 217 (70.9%) | 217 (69.8%) | |
| NSTEMI, n (%) | 84 (13.6%) | 39 (12.7%) | 45 (14.5%) | |
| STEMI, n (%) | 99 (16.0) | 50 (16.3%) | 49 (15.8%) | |
| Laboratory test results | | | | |
| PLT count, 109/L | 215 (182,251) | 215 (179,255) | 215 (184,246) | 0.930 |
| FIB, g/L, M (Q1, Q3) | 2.67 (2.31,3.20) | 2.67 (2.28,3.15) | 2.66 (2.33,3.26) | 0.491 |
| LDL-C, mmol/L, M (Q1, Q3) | 2.08 (1.69,2.66) | 2.05 (1.69,2.52) | 2.16 (1.67,2.73) | 0.310 |
| Cr, μmol/L, M (Q1, Q3) | 72.60 (62.71,83.28) | 72.53 (62.25,83.26) | 72.80 (63.00,83.40) | 0.654 |
| ALT, IU/L, M (Q1, Q3) | 20.80 (15.05,29.52) | 19.95 (14.80,29.00) | 21.10 (15.75,30.40) | 0.121 |

$\bar{x} \pm s$: mean ± standard deviation; M (Q1,Q3), median (interquartile range); UA, unstable angina pectoris; NSTEMI, non-ST-segment elevation myocardial infarction; STEMI, ST-segment elevation myocardial infarction; PLT, platelet; FIB, fibrinogen concentration; LDL-C, low-density lipoprotein cholesterol; Cr, creatinine; ALT, alanine aminotransferase.

tribution were expressed as M (Q1, Q3), and rank sum test was used for inter-group comparison. Enumeration data were expressed in proportion (%), and chi-square test was used for inter-group comparison. Univariate and multivariate Logistic regression was used to analyze the factors affecting the endpoint. When the *p*-value of the bilateral test was less than 0.05, the difference was statistically significant.

## Results

### Demographic and Clinical Data

In this study, 639 patients were enrolled according to inclusion and exclusion criteria, and 617 patients were finally included for analysis (7 patients dropped out due to self-changing medication regimen, 3 patients quit due to surgery, and 12 cases were lost to follow-up). Among them, 434 (70.3%) were males with a median age of 64 years. There were 306 patients in CA group and 311 patients in IA group. The basic clinical data of the patients in the two groups: age, sex, smoking history, drinking history, hypertension, diabetes mellitus, admitting diagnosis, platelet count, fibrinogen, low density lipoprotein cholesterol (LDL-C), creatinine, and alanine aminotransferase showed no statistical difference (all have $p > 0.05$, Table I).

### *CYP2C19* Genotype Distribution in IA Group

There were 311 patients in IA group, 150 (48.2%), 117 (37.6%) and 44 (14.2%) of whom were EMs, IMs, and PMs, respectively (Figure 2). The proportion of patients carrying *CYP2C19*

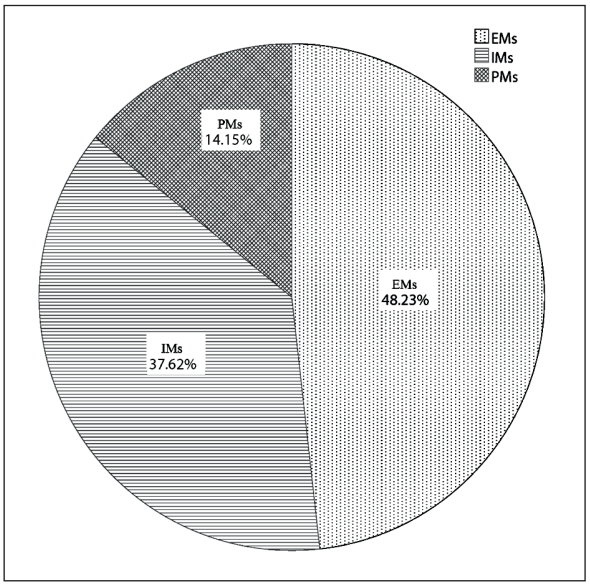


**Figure 2.** Phenotype distribution proportion of *CYP2C19* allele in IA group. IA, individualized antiplatelet therapy group; EMs, extensive metabolizers; IMs, intermediate metabolizers; PMs, poor metabolizers.

LOF alleles was 51.8%. The mutation rate of *CYP2C19* * 2 and * 3 alleles was 33.2%, which was similar to the previous reports on the mutation rate of *CYP2C19**2 and *3 alleles in Chinese Han population[29,30].

### Comparison of Platelet Function Between CA and IA Groups

The patients with $MA_{ADP}$ > 47mm were 89 (29.1%) in the CA group, 89 (28.6%) in the IA group, 50 (33.3%) in the EMs, 36 (30.8%) in the IMs and 3 (6.8%) in the PMs. The patients with $MA_{ADP} \leq 31$ mm were 87 (28.4%) in the CA group, 97 (31.2%) in the IA group, 38 (25.3%) in the EMs, 28 (23.9%) in the IMs and 31 (70.5%) in the PMs.

There was no significant difference in $MA_{ADP}$ classification (≤ 31 mm, 31 mm < $MA_{ADP} \leq 47$ mm, > 47 mm) between CA group and IA group ($p = 0.740$). There was no significant difference between EMs and IMs ($p = 0.808$). There were significant differences between PMs and EMs, PMs and IMs ($p < 0.001$), (Figure 3).

There was no significant difference in the value of $MA_{ADP}$ between CA group and IA group ($p = 0.341$). There was no significant difference in the value of $MA_{ADP}$ among the CA group, IA group, EMs, IMs (all had $p > 0.05$). There were significant differences between PMs and CA group, PMs and EMs, PMs and IMs (all had $p < 0.001$), (Figure 4).

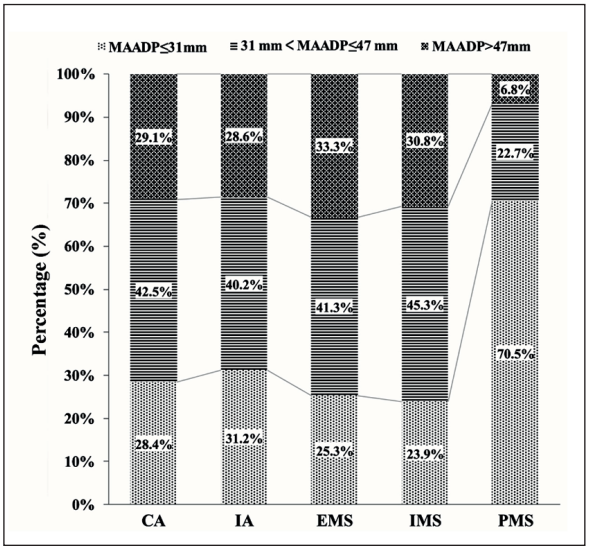


**Figure 3.** Percentage of patients with $MA_{ADP}$ > 47 mm, 31 mm < $MA_{ADP} \leq 47$ mm or $MA_{ADP} \leq 31$ mm in CA, IA groups, EMs, IMs and PMs genotypes. $MA_{ADP}$, ADP-induced platelet-fibrin clot strength; CA, conventional antiplatelet therapy group; IA, individualized antiplatelet therapy group; EMs, extensive metabolizers; IMs, intermediate metabolizers; PMs, poor metabolizers.

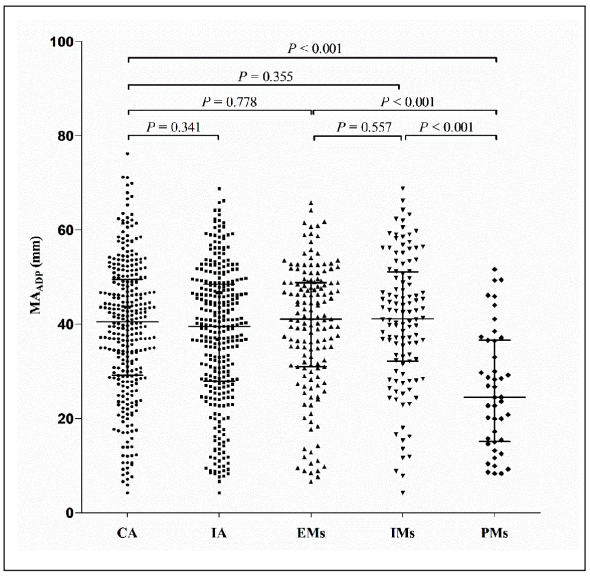


**Figure 4.** $MA_{ADP}$ of patients in CA, IA Groups, EMs, IMs and PMs genotypes. $MA_{ADP}$, ADP-induced platelet-fibrin clot strength; CA, conventional antiplatelet therapy group; IA, individualized antiplatelet therapy group; EMs, extensive metabolizers; IMs, intermediate metabolizers; PMs, poor metabolizers; Lines at median with interquartile range.

In the IA group, there were 50 EMs patients with $MA_{ADP}$ > 47 mm (33.3%). Of which, there were 2 cases who changed the dosage of clopidogrel to 75 mg BID, 14 cases who changed clopidogrel to ticagrelor, 1 case who changed clopidogrel to ticagrelor then changed back to clopidogrel because of the adverse effects of dyspnea of ticagrelor, and the other 33 cases maintained the original treatment. There were 36 IMs patients with $MA_{ADP}$ > 47 mm (30.8%). Of which, there were 19 cases who changed clopidogrel to ticagrelor, 1 case who changed clopidogrel to ticagrelor then changed back to clopidogrel because of outpatient health insurance medication restrictions, and the other 16 cases maintained the original treatment, (Figures 1 and 5).

We compared the $MA_{AA}$ values of Ca and IA groups. $MA_{AA}$ is the strength of platelet fibrin blood clot induced by cyclooxygenase. The larger the value, the worse the response to aspirin. The $MA_{AA}$ values of the two groups did not conform to the normal distribution. By nonparametric test, there was no significant statistical difference in $MA_{AA}$ value between Ca and IA groups ($p = 0.402$; Figure 6).

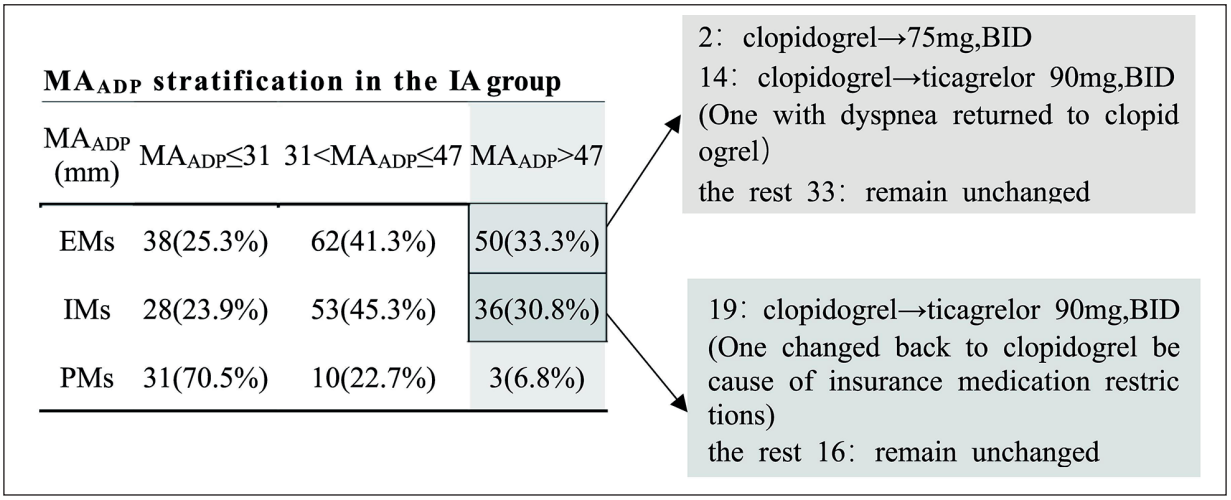


**Figure 5.** Adjust medication according to platelet function MA$_{ADP}$, ADP-induced platelet-fibrin clot strength; CA, conventional antiplatelet therapy group; IA, individualized antiplatelet therapy group; EMs, extensive metabolizers; IMs, intermediate metabolizers; PMs, poor metabolizers.

### *Comparison of Main Efficacy Endpoints Between CA and IA Groups*

Within one year after PCI, 23 (7.5%) cases of MACE occurred in the CA Group which include 16 cases (5.2%) of non-fatal myocardial infarction, 2 (0.7%) cases of stent thrombosis, and 6 (2.0%) cases of all-cause death. Among them, 1 case of myocardial infarction was caused by stent thrombosis, which was counted as 1 MACE to ensure no repeat count.

There were 9 (2.9%) cases of MACE in IA group which include 5 (1.6%) cases of non-fatal myocardial infarction, 1 (0.3%) case of in-stent thrombosis, 4 (1.3%) cases of all-cause death. Among them, 1 case died after stent thrombosis, which was counted as 1 MACE to ensure no repeat count (Figure 7).

The occurrence of MACE in IA group was significantly lower than that in CA Group ($\chi^2$ =

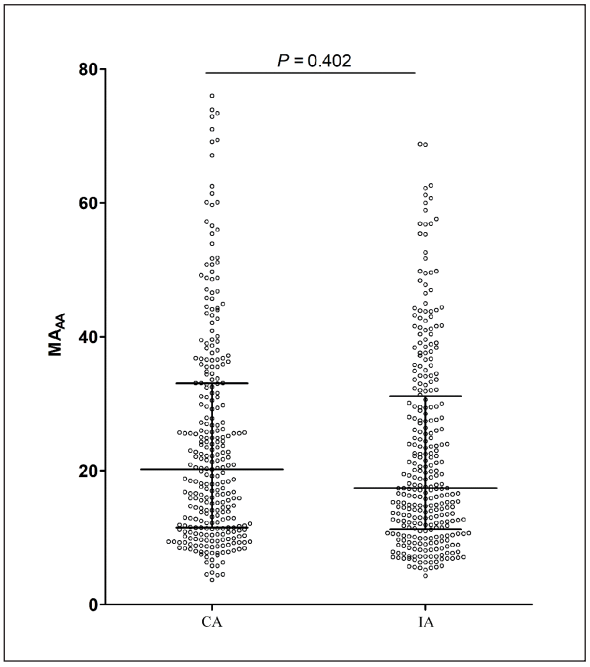


**Figure 6.** MA$_{AA}$ of patients in CA, IA Groups. MA$_{AA}$, cyclooxygenase-induced platelet-fibrin clot strength; CA, conventional antiplatelet therapy group; IA, individualized antiplatelet therapy group.

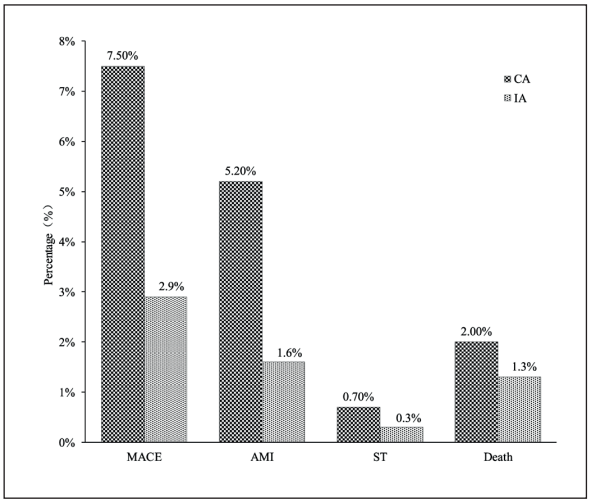


**Figure 7.** Comparison of primary efficacy endpoints between CA and IA groups. CA, conventional antiplatelet therapy group; IA, individualized antiplatelet therapy group; MACE, major adverse cardiac events; AMI, acute myocardial infarction; ST, stent thrombosis.

6.702, $p$ = 0.010). The occurrence of non-fatal myocardial infarction in IA group was significantly lower than that in CA Group ($\chi^2$ = 6.151, $p$ = 0.013). There was no significant difference in the occurrence of in-stent thrombosis between CA and IA Group ($\chi^2$ = 0.351, $p$ = 0.553). There was no significant difference in the occurrence of all-cause death between CA Group and IA group ($\chi^2$ = 0.440, $p$ = 0.507) (Figure 7).

### Comparison of Safety Endpoints Between CA and IA Groups

Within one year after PCI, 7 patients in CA Group and 9 patients in IA group had moderate or severe hemorrhage events. There was no significant difference between the two groups ($\chi^2$ = 0.224, $p$ = 0.636). Within one year after PCI, 7 patients (2.3%) in CA Group and 25 patients (8.0%) in IA group had mild hemorrhage. The rate of slight hemorrhage in IA group was significantly higher than that in CA Group. ($\chi^2$ = 10.374, $p$ = 0.001) (Figure 8).

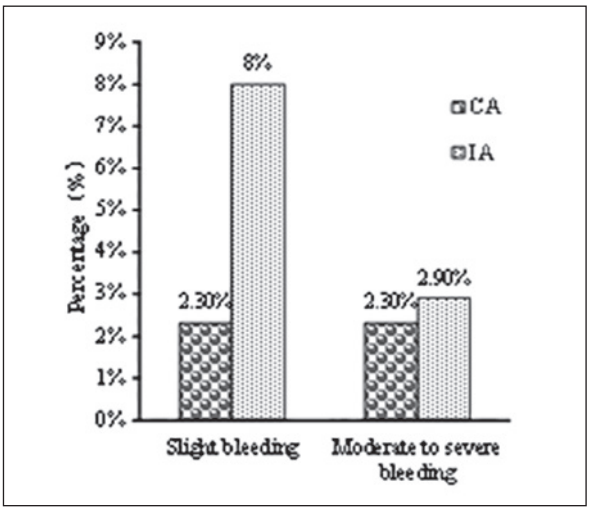


**Figure 8.** Comparison of safe endpoints between CA and IA groups. CA, conventional antiplatelet therapy group; IA, individualized antiplatelet therapy group.

### Logistic Regression Analysis

According to the common risk factors of cardiovascular disease reported in the literature and the distribution characteristics of the population in this study, we took the occurrence of MACE as the dependent variable, selected sex, age, hypertension, diabetes, history of smoking, and $MA_{ADP}$ value as independent variables for univariate logistic regression analysis. The results showed that age ($p$ = 0.003) and diabetes ($p$ = 0.075) may be related to the prevalence of MACE (Table II). Then, with the prevalence of MACE as the dependent variable, age was included in Multivariate Logistic regression analysis (method: forward, likelihood ratio test of conditional parameter estimation). The results showed that age ($p$ = 0.001) was risk factor for MACE in patients with ACS within 1 year after PCI (Table III).

According to the common risk factors of cardiovascular disease reported in the literature and the distribution characteristics of the population in this study, we took the occurrence of non-fatal myocardial infarction as the dependent variable and selected sex, age, hypertension, diabetes, smoking history and $MA_{ADP}$ value as independent variables for univariate logistic regression analysis. The results showed that diabetes ($p$ = 0.016) and $MA_{ADP}$ value ($p$ = 0.081) may be related to the occurrence of non-fatal myocardial infarction (Table IV). Taking whether non-fatal myocardial infarction occurred as the dependent variable, diabetes and the value of $MA_{ADP}$ were included in the multivariate logistic regression analysis (method: forward, likelihood ratio test of conditional parameter estimation). The results showed that diabetes ($p$ = 0.011) and the value of $MA_{ADP}$ ($p$ = 0.003) were the risk factors of non-fatal myocardial infarction (Table V).

**Table II.** Univariate Logistic regression analysis of influencing factors for MACE in patients post PCI within one year.

| Factors | β | S.E, | Wals | df | p | OR (95% CI) |
|---|---|---|---|---|---|---|
| Sex | 0.428 | .487 | .773 | 1 | 0.379 | 1.534 (0.591, 3.983) |
| Age | 0.058 | .020 | 8.626 | 1 | 0.003 | 1.06 (1.019, 1.101) |
| Hypertension | 0.159 | .558 | .081 | 1 | 0.776 | 1.172 (0.393, 3.499) |
| DM | 0.669 | .375 | 3.179 | 1 | 0.075 | 1.953 (0.936, 4.076) |
| Smoking | 0.386 | .474 | .665 | 1 | 0.415 | 1.472 (0.581, 3.726) |
| $MA_{ADP}$ | 0.003 | .032 | .010 | 1 | 0.92 | 1.003 (0.943, 1.067) |

MACE, major adverse cardiac events; PCI, percutaneous coronary intervention; DM, Diabetes mellitus; $MA_{ADP}$, ADP-induced platelet-fibrin clot strength.

**Table III.** Multivariate Logistic regression analysis of influencing factors for MACE in patients post PCI within one year.

| Factors | β | S.E, | Wals | df | p | OR (95% CI) |
|---|---|---|---|---|---|---|
| Age | .060 | .019 | 10.227 | 1 | .001 | 1.062 (1.024, 1.102) |

MACE, major adverse cardiac events; PCI, percutaneous coronary intervention; DM, Diabetes mellitus; $MA_{ADP}$, ADP-induced platelet-fibrin clot strength.

**Table IV.** Multivariate Logistic regression analysis of influencing factors for MACE in patients post PCI within one year.

| Factors | β | S.E, | Wals | df | p | OR (95% CI) |
|---|---|---|---|---|---|---|
| Sex | .525 | .633 | .687 | 1 | .407 | 1.69 (0.489, 5.842) |
| Age | .033 | .024 | 1.925 | 1 | .165 | 1.034 (0.986, 1.083) |
| Hypertension | -.051 | .656 | .006 | 1 | .939 | 0.951 (0.263, 3.440) |
| DM | 1.197 | .497 | 5.789 | 1 | .016 | 3.309 (1.248, 8.773) |
| Smoking | .598 | .616 | .940 | 1 | .332 | 1.818 (0.543, 6.083) |
| $MA_{ADP}$ | .067 | .038 | 3.035 | 1 | .081 | 1.069 (0.992, 1.153) |

AMI, acute myocardial infarction; PCI, percutaneous coronary intervention; DM, Diabetes mellitus; $MA_{ADP}$, ADP-induced platelet-fibrin clot strength.

## Discussion

Our results showed that: There was no significant difference in $MA_{ADP}$ classification ($\leq$ 31 mm, 31 mm < $MA_{ADP} \leq$ 47 mm, > 47 mm) between CA group and IA group ($p$ = 0.740). There was no significant difference in $MA_{ADP}$ classification between EMs and IMs ($p$ = 0.808). There were significant differences in $MA_{ADP}$ classification between PMs and EMs, PMs and IMs ($p < 0.001$) (Figures 3 and 4). (2) The occurrence of MACE, non-fatal myocardial infarction in the IA group were significantly lower than those in the CA group ($p$ = 0.010; $p$ = 0.013). There was no significant difference in the occurrence of in-stent thrombosis and all-cause death between the CA group and the IA group ($p$ = 0.553; $p$ = 0.507) (Figure 7). (3) Within 1 year after PCI, there was no statistically significant difference in the incidence of moderate or severe hemorrhage between the CA group and the IA group ($p$ = 0.636) (Figure 8). (4) Multiple Logiatic regression analysis showed that age ($p$ = 0.001) was the risk factor for MACE within 1 year after PCI in ACS patients, while history of diabetes ($p$ = 0.011) and $MA_{ADP}$ value ($p$ = 0.015) were risk factors for non-fatal myocardial infarction within 1 year after PCI in ACS patients.

### Individualized Antiplatelet Therapy and Platelet Function

In this study, there was no significant difference in the $MA_{ADP}$ grades between EMs and IMs patients. This suggests that individualized treatment with a double dose of clopidogrel in IMs patients achieved similar antiplatelet effects as the regular dose of clopidogrel in EMs patients. This confirmed that a patient with *CYP2C19* LOF allele taking double dose of clopidogrel could overcome the decreased efficacy of clopidogrel associated with *CYP2C19* LOF allele and did not increase the risk of hemorrhage. This is consistent with the results of Mega et al[18] and Collet et al[31].

Clopidogrel *CYP2C19* LOF allele has a gene dose effect: patients with two *CYP2C19* LOF al-

**Table V.** Multivariate Logistic regression analysis of influencing factors for AMI in patients post PCI within one year.

| Factors | β | S.E, | Wals | df | p | OR (95% CI) |
|---|---|---|---|---|---|---|
| DM | 1.255 | .494 | 6.451 | 1 | .011 | 3.507 (1.332, 9.234) |
| $MA_{ADP}$ | .055 | .018 | 8.861 | 1 | .003 | 1.056 (1.019, 1.095) |

AMI, acute myocardial infarction; PCI, percutaneous coronary intervention; DM, Diabetes mellitus; $MA_{ADP}$, ADP-induced platelet-fibrin clot strength.

leles have a higher incidence of HPR than patients with one *CYP2C19* LOF allele[32,33]. Moreover, patients with different numbers of *CYP2C19* LOF alleles also had different responses to the increase of clopidogrel: Mega et al[18] and Collet et al[31] showed that increasing the dose of clopidogrel could increase the platelet inhibition of patients with one *CYP2C19* LOF allele and overcome clopidogrel resistance, but for patients with two *CYP2C19* LOF alleles, the effect of increasing the dose of clopidogrel was not obvious. Based on this, we estimated that increasing the dose of clopidogrel is less likely to overcome the resistance to clopidogrel in PMs patients. So, in this study, we gave ticagrelor combined with aspirin to PMs patients, not clopidogrel. The results showed that the platelet inhibition effect in PMs patients was significantly stronger than that in CA group, EMs and IMs patients taking double dose of clopidogrel. While at the same time, the proportion of patients with lower platelet function also increased significantly in PMs patients. This suggests that the risk of hemorrhage may also increase. However, this is only a conclusion drawn from the detection of platelet function after medication. Further studies are needed to determine whether the risk of clinical hemorrhage will be affected.

### Individualized Antiplatelet Therapy and Prognosis

Comparison of efficacy endpoints in this study showed that within 1 year after PCI, the incidence of MACE and non-fatal myocardial infarction in the IA group was significantly lower than that in the CA group. This is consistent with the following findings: Xie et al[34] (prospective randomized study) conducted individualized antiplatelet therapy based on *CYP2C19* phenotype. The EMs patients and the conventional group received the conventional dose of clopidogrel. The IMs patients took a 600 mg clopidogrel load before PCI and a clopidogrel 150 mg/day maintenance dose after PCI for at least 6 months. The PMs patients received 600 mg clopidogrel and 200 mg cilostazol load before PCI, followed by 100 mg cilotazol bid and clopidogrel 150 mg/day maintenance dose for at least 6 months after PCI. After 6 months, the occurrence of major adverse cardiac or cerebrovascular events in the individualized treatment group was significantly lower than that in the conventional group. Cavallari et al[35] (retrospective) found that the occurrence of MACE within 1 year after PCI in *CYP2C19* LOF carriers treated with clopidogrel was significantly higher than that in patients with alternative therapy (ticagrelor or prasugrel), while in non-*CYP2C19* LOF carriers, there was no significant difference in the occurrence of adverse events between clopidogrel and replacement therapy. In the PHARMCLO[36] study (prospective randomized study), clinicians in the pharmacogenomics group chose $P2Y_{12}$ receptor antagonists (clopidogrel, prasugrel, ticagrelor) base on both the patient's clinical characteristics and genotyping results, while clinicians in the standard treatment group only based on the clinical characteristics of the patients. Analysis of the clinical outcomes of 12 ± 1 month of patients included in the trial showed that the prevalence of ischemic and hemorrhage events in the pharmacogenomics group was significantly lower than that in the standard treatment group. These findings suggest that it is valuable and feasible to adjust the individualized antiplatelet therapy of ACS patients according to the *CYP2C19* genotype to reduce the prevalence of adverse events. Bonello et al[37,38], Siller-Matula et al[39] and Christ et al[40] suggest that patients taking clopidogrel can overcome the increase of the prevalence of ischemic adverse events associated with *CYP2C19* LOF allele through individualized antiplatelet therapy guided by platelet function.

However, some studies have not found that adjusting antiplatelet therapy according to platelet reactivity can reduce the occurrence of cardiovascular adverse events. In the GRAVITAS study of Price et al[41], 2214 patients with stable angina or non ST elevation acute coronary syndrome after PCI detected as HPR by VerifyNow were randomly assigned to take the standard dose of clopidogrel (75 mg/day, n = 1105) or high dose of clopidogrel (150 mg/day, n = 1109). After 6 months of follow-up, it was found that for patients with HPR after PCI, double dose of clopidogrel could reduce the platelet reactivity in patients taking clopidogrel, but could not reduce the occurrence of death due to cardiovascular disease, non fatal myocardial infarction and stent thrombosis. Researchers analyzed that the reasons for not obtaining a positive result include: 1. Compared with the standard dose of clopidogrel, high-dose clopidogrel only reduced platelet reactivity and the ratio of HPR after treatment to an insufficient level. Inadequate treatment may explain the negative results of the study. High-dose clopidogrel may have only a slight pharmacodynamic effect in *CYP2C19* LOF allele carriers, especially in homozygous patients. Application of other

more potent platelet inhibitors such as prasugrel may be more beneficial. 2. The relative benefit of high-dose clopidogrel may also be attenuated by the decrease in the occurrence of HPR in the first 30 days after PCI in both groups. Thirty-eight percent of patients in the standard-dose group who tested as HPR 12 to 24 hours after PCI did not show HPR during 30-day follow-up. In some patients, platelet activation after stent implantation resulted in HPR, which may be the reason for the decrease of platelet reactivity in the early stage after PCI. There is a dynamic change in platelet reactivity itself in patients after PCI, which suggests the importance of timing for measuring platelet reactivity and its relationship with adverse outcomes. The researchers believe that repeated platelet function testing may be more valuable. Because the number of events observed is significantly less than expected, which reduces the effectiveness of the test, the researchers believe that negative test results cannot negate the effect of high-dose clopidogrel. We agree with the author's analysis. The ARCTIC study of Collet et al[42] included 2440 patients who intended to undergo PCI (except acute ST segment elevation myocardial infarction) and were randomly divided into adjustment treatment group and control group. Platelet reactivity was detected by VerifyNow before stent implantation in the treatment group. Patients with aspirin-related HPR were given intravenous aspirin. Patients with clopidogrel-related HPR were given glycoprotein IIb/IIIa inhibitor and additional load dose of clopidogrel (≥ 600 mg) or prasugrel (loading dose 60 mg) before operation, and the daily maintenance dose postoperatively was 150 mg clopidogrel or 10 mg prasugrel. Non-HPR patients and the control group were routinely treated with aspirin, clopidogrel or prasugrel. Platelet activity was measured again 14 to 30 days after stent implantation. Patients with HPR associated with clopidogrel switched to 10 mg of prasugrel or increased the maintenance of clopidogrel by 75 mg. Patients with low platelet reactivity (platelet inhibition rate >90%) during thienopyridine treatment switched to a maintenance dose of 75 mg clopidogrel if they were receiving 10 mg prasugrel or 150 mg clopidogrel. After 1 year of follow-up, the adjustment of antiplatelet therapy according to platelet function monitoring before and after stent implantation failed to reduce the incidence of cardiovascular events compared with conventional treatment strategies that did not monitor the effects of antiplatelet drugs. In the ARCTIC study, platelet reactivity was monitored before and 2 to 4 weeks after PCI, and antiplatelet therapy was adjusted twice. In this trial, the $P2Y_{12}$ receptor inhibitor was mainly clopidogrel, and prasugrel was rarely used (9.3% in adjustment treatment group at the first adjustment and 12.1% after the second adjustment). We think that the reasons for the negative results of the ARCTIC study may be related to the following two points: (1) The HPR patients carrying two *CYP2C19* LOF alleles may not be able to overcome the *CYP2C19* LOF allele-related decrease in the antiplatelet efficacy of clopidogrel by simply increasing the dose of clopidogrel; (2) The patients who took prasugrel and showed low platelet reactivity at the 2nd platelet test may not obtain sufficient platelet inhibition after switching to clopidogrel.

There are many clinical factors that affect the effectiveness of individualized antiplatelet therapy. The effect of individualized treatment is related to the choice of specific individualized treatment options, the choice of patient population, and the choice of prognostic indicators. The interpretation of relevant research should comprehensively analyze the specific research content, methods and the results of each study.

Due to the low prevalence of stent thrombosis and death after PCI, the sample size needed to meet the statistical efficacy of 80% or more is very large. The sample size of this study is insufficient.

This study failed to find the difference in the prevalence of in-stent thrombosis and all-cause death between the CA group and the IA group, which could not be ruled out to be related to the relative insufficient sample size. More trials are needed to confirm the effect of individualized antiplatelet therapy on in-stent thrombosis and the incidence of all-cause death.

### Formulation of Individualized Antiplatelet Therapy

Several studies have shown that the inhibition of platelet aggregation of clopidogrel can be enhanced by increasing the loading or maintenance dose of clopidogrel. Clopidogrel with a maintenance dose of 150 mg had a stronger inhibitory effect on platelet aggregation in most patients than with a maintenance dose of 75 mg[43,44]. Individualized treatment with up to four repeated loading doses of clopidogrel or single reloading and double maintenance doses of clopidogrel overcomes the HPR when taking conventional doses of clopidogrel[37-39].

Clopidogrel *CYP2C19* LOF allele has gene dose effect: patients with two *CYP2C19* LOF alleles have higher HPR occurrence than patients with one *CYP2C19* LOF allele[32]. Moreover, patients with different amounts of *CYP2C19* LOF gene responded differently to the increase of clopidogrel dose[33]. Increasing the dose of clopidogrel can increase the platelet inhibition of patients carrying one *CYP2C19* LOF gene and overcome the resistance of clopidogrel; but for patients carrying two *CYP2C19* LOF genes, the effect of increasing the dose of clopidogrel is not evident[18,31].

Therefore, in this study, IMs patients with one *CYP2C19* LOF allele and PMs patients with two *CYP2C19* LOF alleles were given different individualized antiplatelet therapy.

Current research on individualized antiplatelet therapy is either based on *CYP2C19* phenotype or platelet reactivity. Most of them adjust individualized antiplatelet therapy based on a single basis. In this study, according to *CYP2C19* genotyping and platelet function, two pieces of information were applied to the adjustment of antiplatelet therapy, and the positive results were obtained. Although there is a good correlation between *CYP2C19* LOF allele carrying and platelet reactivity when treated with clopidogrel, they do not completely overlap. *CYP2C19**2 carrier status explained only 12% of platelet aggregation variation after medication, while other factors accounted for more than 80% of the variation[45]. If antiplatelet drugs are adjusted solely on the basis of genotyping, a considerable number of clopidogrel non-responders will be missed[33]. Adjusting antiplatelet therapy based on genotyping alone is not the best strategy. If antiplatelet therapy is only guided by platelet function, due to the dose effect of the *CYP2C19* LOF allele, patients who are detected as HPR will have different responses to the adjusted treatment plan according to their genotyping, which will weaken the effect of individualized therapy to a great extent. Moreover, ACS patients need to get effective and sufficient antiplatelet therapy as soon as possible. When adjusting the treatment according to the platelet reactivity, it is necessary for the patients to take antiplatelet drugs to reach the steady state and then detect the platelet function. In this way, for patients who have not taken the related antiplatelet drugs before, if the treatment plan is only adjusted according to the platelet reactivity, the time for adjusting the plan shall be at least hours after taking the medicine. Due to the development and progress of detection technology, rapid bedside genotyping has been able to provide genotypic results within 70 minutes after blood sampling. Individual antiplatelet therapy based on genotyping can adjust the drug of *CYP2C19* LOF gene carriers who are more likely to have HPR as soon as the results of genotyping are returned. The combination of genotyping and platelet function to guide individualized antiplatelet therapy should be a better choice.

We designed two steps to adjust antiplatelet treatment to achieve adequate and safe antiplatelet therapy: first, immediately after the patients entered the IA group, we conducted individualized antiplatelet therapy according to *CYP2C19* genotype. Then, after the drug reached a relatively stable blood concentration *in vivo*, we measured the residual platelet activity with antiplatelet therapy using TEG and adjusted the antiplatelet therapy in patients showed HPR. In this study, individualized treatment was started immediately after *CYP2C19* genotype was detected, rather than after the administration of antiplatelet medicine and platelet function detection. Thus, the time point to start individualized intervention is advanced, and the second adjustment fulfilled further optimization of antiplatelet therapy. At the same time, in this study, the patients with one or two LOF alleles were given different individualized antiplatelet therapy in view of their different response to the enhanced antiplatelet therapy. It not only helps patients with two *CYP2C19* LOF alleles to have sufficient antiplatelet therapy, but also avoids the increase of hemorrhage risk in patients with one *CYP2C19* LOF allele.

Our results suggest that individualized antiplatelet therapy can significantly reduce the prevalence of MACE (mainly the prevalence of acute non-fatal myocardial infarction) after PCI, while the prevalence of moderate to severe hemorrhage has no significant change.

In this study, logistic regression analysis indicated that the value of $MA_{ADP}$ was a risk factor of non fatal myocardial infarction. It is verified that the inhibition of platelet function after antiplatelet therapy is related to the occurrence of myocardial infarction after PCI. It provides the basis for adjusting antiplatelet therapy according to $MA_{ADP}$.

The results of this study failed to find that the value of $MA_{ADP}$ is related to the occurrence of MACE. The reason may be that MACE defined in this study includes stent thrombosis and all-cause

death, except for fatal myocardial infarction: stent thrombosis is not only related to platelet function, but also related to factors such as too large or too small stent, poor stent attachment, mechanical damage to the vessel wall during intervention, and all-cause death is associated with many non-cardiovascular factors.

In summary, our results indicate that: 1. Patients with a *CYP2C19* LOF gene who take double doses of clopidogrel overcome the decreased efficacy of clopidogrel which partly due to *CYP2C19* LOF gene, without increasing the risk of hemorrhage 2. Individualized antiplatelet therapy based on *CYP2C19* genotype and platelet function significantly reduced the occurrence of MACE after PCI without increasing the risk of moderate or severe hemorrhage. For ACS patients who cannot tolerate tegregrel or choose clopidogrel for social and economic reasons, especially for ACS patients after PCI, this study provides a more effective and safe individualized treatment scheme for the selection and application of $P2Y_{12}$ receptor inhibitors and provides a theoretical basis for further optimization of antiplatelet therapy.

Due to the low occurrence of stent thrombosis and death after PCI, the sample size needed is very large, so the sample size of this test is relatively insufficient when the two factors are statistically analyzed, and the exact conclusions related to these two factors cannot be obtained. The CA Group in this study did not carry out *CYP219* genotype test and the genotype could not be included in the risk factor analysis, which may lead to some errors in the statistical results.

## Conclusions

Shortly, patients with a *CYP2C19* LOF gene who take double doses of clopidogrel overcome the decreased efficacy of clopidogrel which partly due to *CYP2C19* LOF gene, without increasing the risk of hemorrhage. 2. Individualized antiplatelet therapy based on *CYP2C19* genotype and platelet function can significantly reduce the occurrence of MACE (mainly acute non-fatal myocardial infarction) after PCI without increasing the risk of moderate and severe hemorrhage.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

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## References

1) Davi G, Patrono C. Platelet activation and atherothrombosis. N Engl J Med 2007; 357: 2482-2494.
2) Kereiakes DJ, Gurbel PA. Peri-procedural platelet function and platelet inhibition in percutaneous coronary intervention. JACC Cardiovasc Interv 2008; 1: 111-121.
3) Roffi M, Patrono C, Collet JP, Mueller C, Valgimigli M, Andreotti F, Bax JJ, Borger MA, Brotons C, Chew DP, Gencer B, Hasenfuss G, Kjeldsen K, Lancellotti P, Landmesser U, Mehilli J, Mukherjee D, Storey RF, Windecker S, Group ESCSD. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). Eur Heart J 2016; 37: 267-315.
4) Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, Caforio ALP, Crea F, Goudevenos JA, Halvorsen S, Hindricks G, Kastrati A, Lenzen MJ, Prescott E, Roffi M, Valgimigli M, Varenhorst C, Vranckx P, Widimsky P, Group ESCSD. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). Eur Heart J 2018; 39: 119-177.
5) Amsterdam EA, Wenger NK, Brindis RG, Casey DE, Jr., Ganiats TG, Holmes DR, Jr., Jaffe AS, Jneid H, Kelly RF, Kontos MC, Levine GN, Liebson PR, Mukherjee D, Peterson ED, Sabatine MS, Smalling RW, Zieman SJ. 2014 AHA/ACC Guideline for the Management of Patients with Non-ST-Elevation Acute Coronary Syndromes: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 2014; 64: e139-e228.
6) China Society of Cardiology of Chinese Medical Association, Editorial Board of Chinese Journal of Cardiology. Guideline on the diagnosis and therapy of ST-segment elevation myocardial infarction. Chin J Cardiol 2015; 43: 380-393.

7) Section of Interventional Cardiology of Chinese Society of Cardiology of Chinese Medical Association, Specialty Committee on Prevention and Treatment of Thrombosis of Chinese College of Cardiovascular physicians, Editorial Board of Chinese Journal of Cardiology. Chinese guideline for percutaneous coronary intervention(2016). Chin J Cardiol 2016; 44: 382-400.

8) Chinese Society of Cardiology of Chinese Medical Association, Editorial Board of Chinese Journal of Cardiology. Guideline and Consensus for the management of patients with non-ST-elevation acute coronary syndrome(2016). Chin J Cardiol 2017; 45: 359-376.

9) 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: 2908-2913.

10) Stone GW, Witzenbichler B, Weisz G, Rinaldi MJ, Neumann FJ, Metzger DC, Henry TD, Cox DA, Duffy PL, Mazzaferri E, Gurbel PA, Xu K, Parise H, Kirtane AJ, Brodie BR, Mehran R, Stuckey TD, Investigators A-D. Platelet reactivity and clinical outcomes after coronary artery implantation of drug-eluting stents (ADAPT-DES): a prospective multicentre registry study. Lancet 2013; 382: 614-623.

11) Johnson JA, Cavallari LH. Pharmacogenetics and cardiovascular disease--implications for personalized medicine. Pharmacol Rev 2013; 65: 987-1009.

12) Goodman SG, Nicolau JC, Requena G, Maguire A, Blankenberg S, Chen JY, Granger CB, Grieve R, Pocock SJ, Simon T, Yasuda S, Vega AM, Brieger D. Longer-term oral antiplatelet use in stable post-myocardial infarction patients: Insights from the long Term rIsk, clinical manaGement and healthcare Resource utilization of stable coronary artery dISease (TIGRIS) observational study. Int J Cardiol 2017; 236: 54-60.

13) Kazui M, Nishiya Y, Ishizuka T, Hagihara K, Farid NA, Okazaki O, Ikeda T, Kurihara A. Identification of the human cytochrome P450 enzymes involved in the two oxidative steps in the bioactivation of clopidogrel to its pharmacologically active metabolite. Drug Metab Dispos 2010; 38: 92-99.

14) Hou X, Shi J, Sun H. Gene polymorphism of cytochrome P450 2C19*2 and clopidogrel resistance reflected by platelet function assays: a meta-analysis. Eur J Clin Pharmacol 2014; 70: 1041-1047.

15) Scott SA, Sangkuhl K, Stein CM, Hulot JS, Mega JL, Roden DM, Klein TE, Sabatine MS, Johnson JA, Shuldiner AR. Clinical Pharmacogenetics Implementation Consortium guidelines for CYP2C19 genotype and clopidogrel therapy: 2013 update. Clin Pharmacol Ther 2013; 94: 317-323.

16) Pereira NL, Rihal CS, So DYF, Rosenberg Y, Lennon RJ, Mathew V, Goodman SG, Weinshilboum RM, Wang L, Baudhuin LM, Lerman A, Hasan A, Iturriaga E, Fu YP, Geller N, Bailey K, Farkouh ME. Clopidogrel Pharmacogenetics. Circ Cardiovasc Interv 2019; 12: e007811.

17) Collet JP, Hulot JS, Anzaha G, Pena A, Chastre T, Caron C, Silvain J, Cayla G, Bellemain-Appaix A, Vignalou JB, Galier S, Barthelemy O, Beygui F, Gallois V, Montalescot G, Investigators C-. High doses of clopidogrel to overcome genetic resistance: the randomized crossover CLOVIS-2 (Clopidogrel and Response Variability Investigation Study 2). JACC Cardiovascular interventions 2011; 4: 392-402.

18) Mega JL, Hochholzer W, Frelinger AL, 3rd, Kluk MJ, Angiolillo DJ, Kereiakes DJ, Isserman S, Rogers WJ, Ruff CT, Contant C, Pencina MJ, Scirica BM, Longtine JA, Michelson AD, Sabatine MS. Dosing clopidogrel based on CYP2C19 genotype and the effect on platelet reactivity in patients with stable cardiovascular disease. JAMA 2011; 306: 2221-2228.

19) Cuisset T, Quilici J, Cohen W, Fourcade L, Saut N, Pankert M, Gaborit B, Carrieri P, Morange PE, Bonnet JL, Alessi MC. Usefulness of high clopidogrel maintenance dose according to CYP2C19 genotypes in clopidogrel low responders undergoing coronary stenting for non ST elevation acute coronary syndrome. Am J Cardiol 2011; 108: 760-765.

20) Jeong YH, Kim IS, Park Y, Kang MK, Koh JS, Hwang SJ, Kwak CH, Hwang JY. Carriage of cytochrome 2C19 polymorphism is associated with risk of high post-treatment platelet reactivity on high maintenance-dose clopidogrel of 150 mg/day: results of the ACCEL-DOUBLE (Accelerated Platelet Inhibition by a Double Dose of Clopidogrel According to Gene Polymorphism) study. JACC Cardiovasc Interv 2010; 3: 731-741.

21) Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, Joint ESCAAHAWHFTFftUDoMI, Katus HA, Lindahl B, Morrow DA, Clemmensen PM, Johanson P, Hod H, Underwood R, Bax JJ, Bonow RO, Pinto F, Gibbons RJ, Fox KA, Atar D, Newby LK, Galvani M, Hamm CW, Uretsky BF, Steg PG, Wijns W, Bassand JP, Menasche P, Ravkilde J, Ohman EM, Antman EM, Wallentin LC, Armstrong PW, Simoons ML, Januzzi JL, Nieminen MS, Gheorghiade M, Filippatos G, Luepker RV, Fortmann SP, Rosamond WD, Levy D, Wood D, Smith SC, Hu D, Lopez-Sendon JL, Robertson RM, Weaver D, Tendera M, Bove AA, Parkhomenko AN, Vasilieva EJ, Mendis S. Third universal definition of myocardial infarction. Circulation 2012; 126: 2020-2035.

22) Chinese Society of Cardiology of Chinese Medical Association, Editorial Board of Chinese Journal of Cardiology. Guideline of non-ST segment elevation acute coronary syndrome. Chin J Cardiol 2012; 40: 353-367.

23) Dehmer GJ, Blankenship JC, Cilingiroglu M, Dwyer JG, Feldman DN, Gardner TJ, Grines CL, Singh M. SCAI/ACC/AHA Expert Consensus Document: 2014 update on percutaneous coronary intervention without on-site surgical backup. J Am Coll Cardiol 2014; 63: 2624-2641.

24) Section of Interventional Cardiology of Chinese Society of Cardiology of Chinese Medical Association, Specialty Committee on Prevention and Treatment of Thrombosis of Chinese College of Cardiovascular Phy-

sicians, Editorial Board of Chinese Journal of Cardiology. Chinese guideline for percutaneous coronary intervention(pocket guideline). Chin J Cardiol 2012; 40: 271-277.

25) Chinese Society of Cardiology of Chinese Medical Association, Editorial Board of Chinese Journal of Cardiology. Guideline for diagnosis and treatment of patients with ST-elevation myocardial infarction. Chin J Cardiol 2010; 8: 675-690.

26) Cutlip DE, Windecker S, Mehran R, Boam A, Cohen DJ, van Es GA, Steg PG, Morel MA, Mauri L, Vranckx P, McFadden E, Lansky A, Hamon M, Krucoff MW, Serruys PW, Academic Research C. Clinical end points in coronary stent trials: a case for standardized definitions. 2007; 115: 2344-2351.

27) Mehran R, Rao SV, Bhatt DL, Gibson CM, Caixeta A, Eikelboom J, Kaul S, Wiviott SD, Menon V, Nikolsky E, Serebruany V, Valgimigli M, Vranckx P, Taggart D, Sabik JF, Cutlip DE, Krucoff MW, Ohman EM, Steg PG, White H. Standardized bleeding definitions for cardiovascular clinical trials: a consensus report from the Bleeding Academic Research Consortium. Circulation 2011; 123: 2736-2747.

28) Gurbel PA, Bliden KP, Navickas IA, Mahla E, Dichiara J, Suarez TA, Antonino MJ, Tantry US, Cohen E. Adenosine diphosphate-induced platelet-fibrin clot strength: a new thrombelastographic indicator of long-term poststenting ischemic events. Am Heart J 2010; 160: 346-354.

29) Feng GX, Liang Y, Bai Y, Chen T, Liu X, Yang YM, Wang XY, Yang YJ, Zhu J. Clopidogrel metabolism related gene polymorphisms in Chinese patients with acute coronary syndrome. Chin J Cardiol 2012; 40: 908-913.

30) Tantry US, Bonello L, Aradi D, Price MJ, Jeong YH, Angiolillo DJ, Stone GW, Curzen N, Geisler T, Ten Berg J, Kirtane A, Siller-Matula J, Mahla E, Becker RC, Bhatt DL, Waksman R, Rao SV, Alexopoulos D, Marcucci R, Reny JL, Trenk D, Sibbing D, Gurbel PA, Working Group on On-Treatment Platelet R. Consensus and update on the definition of on-treatment platelet reactivity to adenosine diphosphate associated with ischemia and bleeding. J Am Coll Cardiol 2013; 62: 2261-2273.

31) Collet JP, Hulot JS, Anzaha G, Pena A, Chastre T, Caron C, Silvain J, Cayla G, Bellemain-Appaix A, Vignalou JB, Galier S, Barthelemy O, Beygui F, Gallois V, Montalescot G. High doses of clopidogrel to overcome genetic resistance: the randomized crossover CLOVIS-2 (Clopidogrel and Response Variability Investigation Study 2). JACC Cardiovasc Interv 2011; 4: 392-402.

32) Price MJ, Murray SS, Angiolillo DJ, Lillie E, Smith EN, Tisch RL, Schork NJ, Teirstein PS, Topol EJ, Investigators G. Influence of genetic polymorphisms on the effect of high- and standard-dose clopidogrel after percutaneous coronary intervention: the GIFT (Genotype Information and Functional Testing) study. J Am Coll Cardiol 2012; 59: 1928-1937.

33) Gross L, Trenk D, Jacobshagen C, Krieg A, Gawaz M, Massberg S, Baylacher M, Aradi D, Stimpfle F, Hromek J, Vogelgesang A, Hadamitzky M, Sibbing D, Geisler T. Genotype-phenotype association and impact on outcomes following guided de-escalation of anti-platelet treatment in acute coronary syndrome patients: The TROPICAL-ACS Genotyping Substudy. Thromb Haemost 2018; 118: 1656-1667.

34) Xie X, Ma YT, Yang YN, Li XM, Zheng YY, Ma X, Fu ZY, Ba B, Li Y, Yu ZX, Chen Y, Chen BD, Liu F, Huang Y, Liu C, Baituola G. Personalized antiplatelet therapy according to CYP2C19 genotype after percutaneous coronary intervention: a randomized control trial. Int J Cardiol 2013; 168: 3736-3740.

35) Cavallari LH, Lee CR, Beitelshees AL, Cooper-DeHoff RM, Duarte JD, Voora D, Kimmel SE, McDonough CW, Gong Y, Dave CV, Pratt VM, Alestock TD, Anderson RD, Alsip J, Ardati AK, Brott BC, Brown L, Chumnumwat S, Clare-Salzler MJ, Coons JC, Denny JC, Dillon C, Elsey AR, Hamadeh IS, Harada S, Hillegass WB, Hines L, Horenstein RB, Howell LA, Jeng LJB, Kelemen MD, Lee YM, Magvanjav O, Montasser M, Nelson DR, Nutescu EA, Nwaba DC, Pakyz RE, Palmer K, Peterson JF, Pollin TI, Quinn AH, Robinson SW, Schub J, Skaar TC, Smith DM, Sriramoju VB, Starostik P, Stys TP, Stevenson JM, Varunok N, Vesely MR, Wake DT, Weck KE, Weitzel KW, Wilke RA, Willig J, Zhao RY, Kreutz RP, Stouffer GA, Empey PE, Limdi NA, Shuldiner AR, Winterstein AG, Johnson JA. Multisite investigation of outcomes with implementation of CYP2C19 genotype-guided antiplatelet therapy after percutaneous coronary intervention. JACC Cardiovasc Interv 2018; 11: 181-191.

36) Notarangelo FM, Maglietta G, Bevilacqua P, Cereda M, Merlini PA, Villani GQ, Moruzzi P, Patrizi G, Malagoli Tagliazucchi G, Crocamo A, Guidorossi A, Pigazzani F, Nicosia E, Paoli G, Bianchessi M, Comelli MA, Caminiti C, Ardissino D. Pharmacogenomic approach to selecting antiplatelet therapy in patients with acute coronary syndromes: the PHARMCLO trial. J Am Coll Cardiol 2018; 71: 1869-1877.

37) Bonello L, Camoin-Jau L, Arques S, Boyer C, Panagides D, Wittenberg O, Simeoni MC, Barragan P, Dignat-George F, Paganelli F. Adjusted clopidogrel loading doses according to vasodilator-stimulated phosphoprotein phosphorylation index decrease rate of major adverse cardiovascular events in patients with clopidogrel resistance: a multicenter randomized prospective study. J Am Coll Cardiol 2008; 51: 1404-1411.

38) Bonello L, Camoin-Jau L, Armero S, Com O, Arques S, Burignat-Bonello C, Giacomoni MP, Bonello R, Collet F, Rossi P, Barragan P, Dignat-George F, Paganelli F. Tailored clopidogrel loading dose according to platelet reactivity monitoring to prevent acute and subacute stent thrombosis. Am J Cardiol 2009; 103: 5-10.

39) Siller-Matula JM, Francesconi M, Dechant C, Jilma B, Maurer G, Delle-Karth G, Gouya G, Ruzicka K, Podczeck-Schweighofer A, Christ G. Personalized antiplatelet treatment after percutaneous coronary intervention: the MADONNA study. Int J Cardiol 2013; 167: 2018-2023.

40) Christ G, Siller-Matula JM, Francesconi M, Dechant C, Grohs K, Podczeck-Schweighofer A. Individualising dual antiplatelet therapy after percutaneous coronary intervention: the IDEAL-PCI registry. BMJ Open 2014; 4: e005781.

41) Price MJ, Berger PB, Teirstein PS, Tanguay JF, Angiolillo DJ, Spriggs D, Puri S, Robbins M, Garratt KN, Bertrand OF, Stillabower ME, Aragon JR, Kandzari DE, Stinis CT, Lee MS, Manoukian SV, Cannon CP, Schork NJ, Topol EJ, Investigators G. Standard- vs high-dose clopidogrel based on platelet function testing after percutaneous coronary intervention: the GRAVITAS randomized trial. JAMA 2011; 305: 1097-1105.

42) Collet JP, Cuisset T, Range G, Cayla G, Elhadad S, Pouillot C, Henry P, Motreff P, Carrie D, Boueri Z, Belle L, Van Belle E, Rousseau H, Aubry P, Monsegu J, Sabouret P, O'Connor SA, Abtan J, Kerneis M, Saint-Etienne C, Barthelemy O, Beygui F, Silvain J, Vicaut E, Montalescot G, Investigators A. Bedside monitoring to adjust antiplatelet therapy for coronary stenting. N Engl J Med 2012; 367: 2100-2109.

43) von Beckerath N, Kastrati A, Wieczorek A, Pogatsa-Murray G, Sibbing D, Graf I, Schomig A. A double-blind, randomized study on platelet aggregation in patients treated with a daily dose of 150 or 75 mg of clopidogrel for 30 days. Eur Heart J 2007; 28: 1814-1819.

44) Angiolillo DJ, Bernardo E, Palazuelos J, Desai B, Weisberg I, Alfonso F, Guzman LA, Hernandez-Antolin R, Zenni MZ, Macaya C, Fernandez-Ortiz A, Bass TA. Functional impact of high clopidogrel maintenance dosing in patients undergoing elective percutaneous coronary interventions. Results of a randomized study. Thromb Haemost 2008; 99: 161-168.

45) Hochholzer W, Trenk D, Fromm MF, Valina CM, Stratz C, Bestehorn HP, Buttner HJ, Neumann FJ. Impact of cytochrome P450 2C19 loss-of-function polymorphism and of major demographic characteristics on residual platelet function after loading and maintenance treatment with clopidogrel in patients undergoing elective coronary stent placement. J Am Coll Cardiol 2010; 55: 2427-2434.