

Assessment of Risk Factors for Drug Resistance of Dual Anti Platelet Therapy After PCI

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Lijie Zhang, PhD, MD^{1*}, Ying Lv, PhD, MD^{2*}, Jianyu Dong, PhD, MD^{3*},
Nana Wang, MSc⁴, Zhan Zhan, MSc⁵, Yuan Zhao, BSc¹,
and Shanshan Jiang, PhD, MD¹

Abstract

Since aspirin and clopidogrel are the widely and conventionally used drugs to treat acute myocardial infarction after percutaneous coronary intervention (PCI), it is important to explore potential risk factors of their resistance. The platelet aggregation rate with arachidonic acid (AA, PAg-AA%) and adenosine diphosphate (ADP, PAg-ADP%) of 219 PCI patients were measured after standard treatment for 24 h. The disease history and laboratory data (before PCI) were obtained. We found 101 (46.12%) patients to be aspirin-resistant, and PAg-ADP% was the most prominent risk factor of aspirin resistance. Clopidogrel resistance was present in 157 of 219 patients. Patients in the clopidogrel-resistant group carried more CYP2C19*3 or *2, which was associated with higher clopidogrel resistance in this group (69.11%, 47/68) than in the control group (64.29%, 36/56). Platelet count ($10^9/L$) and hemoglobin (g/L) were the prominent risk factors of clopidogrel resistance. Among the 219 patients, 98 showed dual antiplatelet drug resistance, for which platelet count ($10^9/L$) and monocyte count (g/L) were the risk factors. Aspirin resistance was found to usually accompany clopidogrel resistance.

Keywords

aspirin resistance, clopidogrel resistance, platelet aggregation, cytochrome p-450 CYP2C19, percutaneous coronary intervention

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Introduction

Dual antiplatelet therapy (DAPT), which involves the administration of a combination of aspirin and P2Y₁₂ platelet receptor antagonist, is the main antiplatelet therapy in acute myocardial infarction after percutaneous coronary intervention and has a prominent curative effect.^{1,2} However, in some patients, this therapy may have unexpected effects: DAPT has been reported to increase the risk of bleeding as well as thrombotic risk in some patients.³⁻⁵ Therefore, it is essential to monitor the effect of DAPT. The platelet aggregation rate with arachidonic acid (AA; PAg-AA%) and adenosine diphosphate (ADP; PAg-ADP%) could reflect the effect of aspirin and clopidogrel, respectively, in an individual and could be a predictor of an adverse event: excessive PAg% is indicative of poor drug efficacy and increased risk to thrombotic events, and excessively low PAg% was associated with an increased risk of bleeding.⁶ In this study, we tried to use PAg-AA% and PAg-ADP% to represent the efficacy of DAPT and provide evidence in favor of

individual therapy. The primary aim of this study was to explore the risk factors of DAPT resistance to predict potential aspirin and clopidogrel resistance and to choose effective drugs to reduce bleeding or thrombotic risk of patients after PCI.

¹ Institute of Hematological Research, Shaanxi Provincial People's Hospital, Xi'an, China

² Department of Cardiology, Shaanxi Provincial People's Hospital, Xi'an, China

³ Breast Center, Department of general surgery, Nanfang Hospital, Southern Medical University, Guangzhou, China

⁴ Central laboratory, Shaanxi Provincial People's Hospital, Xi'an, China

⁵ Inspection Center of Hubei Medical Products Administration (Hubei Center for Vaccine Inspection), Wuhan, China

*Lijie Zhang, Ying Lv and Jianyu Dong contributed equally to this work.

Corresponding Author:

Shanshan Jiang, Institute of Hematological Research, Shaanxi Provincial People's Hospital, 256 West Youyi road, Xi'an, Shaanxi 71000, China.
Email: ji.ang.shan.shan@163.com



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Methods and Materials

Study Population

We included 219 patients with acute myocardial infarction who underwent successful PCI in the Department of Cardiology of Shaanxi Provincial People's Hospital between July 2020 and January 2021; their PAg-AA% and PAg-ADP% were tested. Aspirin and clopidogrel resistance were defined as PAg-AA% > 55% and PAg-ADP% > 55% following the manufacturer's instructions of a platelet function analyzer [Aggrestar (PL-12) Platelet Function Analyzer, Sinnowa, Jiangsu, China] and based on another relative study.⁷ Patients with PCI were administered aspirin (100 mg/d) and clopidogrel (75 mg/d); PAg-AA%, PAg-ADP%, maximum aggregation time of AA (MAT-AA, s), and maximum aggregation time of ADP (MAT-ADP, s) were measured after 24 h. Then, according to the PAg-AA% and PAg-ADP% values, these 219 patients were divided into the aspirin-resistant group and control group, into clopidogrel-resistant group and control group, and into aspirin- and clopidogrel-resistant group (dual antiplatelet drug-resistant group) and control group (containing individuals from single-resistant and non-resistant groups). The key exclusion criteria were as follows: patients with a disease that involved a bleeding risk, such as hematological disease and cancer, and patients with severe liver function damage. This study was approved by the Ethics Committee of Shaanxi Provincial People's Hospital and the Ethics Committee of Xi'an Jiaotong University (2018-379).

Data Acquisition

Data on age, sex, and the history of hypertension, hyperlipidemia, diabetes, and stroke were collected. The laboratory test indices assessed before the PCI included complete blood count, D-dimers (mg/L), high-density lipoprotein (mmol/L), low-density lipoprotein (mmol/L), triglyceride (mmol/L), cholesterol (mmol/L), uric acid ($\mu\text{mol/L}$), and glycated hemoglobin (%).

2.3 Measurement of Platelet Function

Herein, PAg-AA%, PAg-ADP%, MAT-AA, and MAT-ADP were tested using an Aggrestar (PL-12) Platelet Function Analyzer: $2 \times 300 \mu\text{L}$ of citrated, whole-blood samples were transferred into supporting tubes, and then, $30 \mu\text{L}$ of AA (2 mg/mL) and $30 \mu\text{L}$ of ADP (50 $\mu\text{mol/L}$) were added to the reagent tubes separately. The AA (2210809) and ADP (2211220) were purchased from Sinnowa (Jiangsu, China). Then, the analyzer tested the platelet count five times and automatically displayed the results with 15 min.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS ver. 17, IBM Corp., Armonk, NY, USA), and the findings for the parameters of hypertension, hyperlipidemia, diabetes, stroke, and sex were compared using the chi-square test, whereas other parameters were compared using the Student's

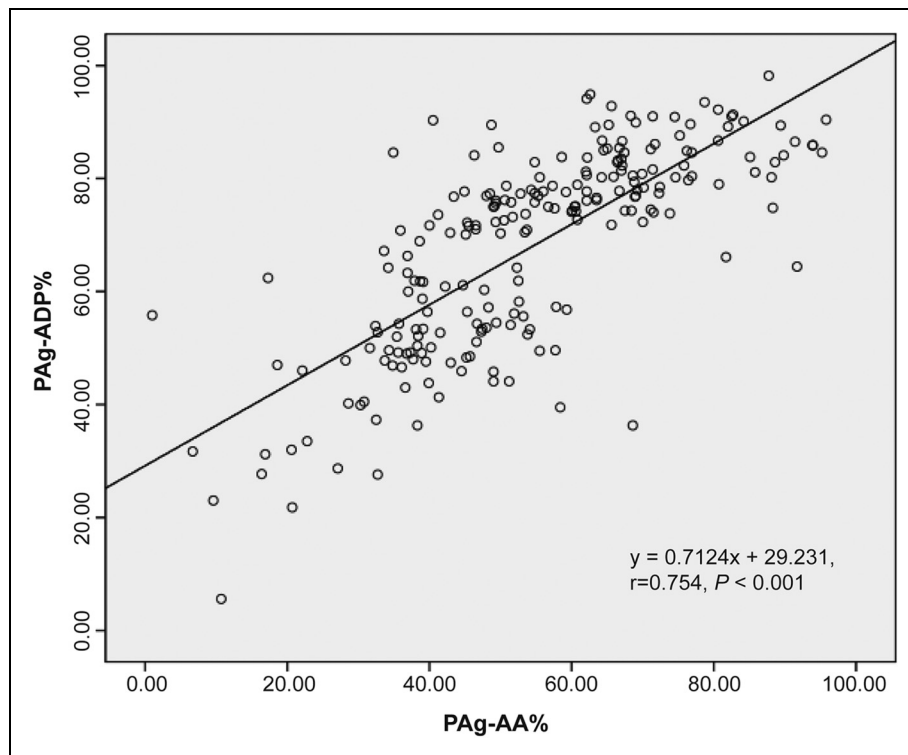


Figure 1. Linear correlation of PAg-AA% and PAg-ADP. Person correlation was performed to test the correlation of PAg-AA% and PAg-ADP. The liner value r has been deduced. The concentration of AA is 2 mg/mL, of ADP is 50 $\mu\text{mol/L}$.

t-test or Mann–Whitney U test. Logistic regression analysis was used to compare the correlation between drug resistance and other factors. The sex, age, hypertension, hyperlipidemia, diabetes, stroke, and laboratory data (eg, PAg-ADP% and monocyte count) were compared by univariate analysis. Along with sex, age, hypertension, hyperlipidemia, diabetes, and stroke, laboratory factors showing $P < 0.1$ in univariate analysis were chosen for multivariate analysis. Pearson correlation was used to investigate the linear correlation between PAg-AA% and PAg-ADP%. $P < 0.05$ was considered statistically significant.

Results

The Clinical Characteristics in Drug Resistance/Control Groups

We selected 219 patients, comprising 166 men and 53 women with the mean age of 61.21 ± 12.29 years. Among them, 101 patients (46.12%, PAg-AA% of 70.70 ± 10.53) belonged to the aspirin-resistant group and 118 (PAg-AA% of 40.42 ± 11.16) to the control group. PAg-ADP% was $79.94 \pm 10.41\%$ in aspirin-resistant group, which was evidently higher than

the control group ($57.64 \pm 1.91\%$, $P < 0.001$). Surprisingly, PAg-AA% and PAg-ADP% appeared to have linear correlation (Figure 1, $r = 0.754$, $P < 0.001$). Moreover, the platelet count was also lower in the aspirin-resistant group ($P < 0.001$). The remaining values, such as MAT-AA, MAT-ADP, complete blood count, glycated hemoglobin, uric acid, D-dimers, sex, age, hypertension, hyperlipidemia, diabetes, and stroke, showed no significant difference between the groups (Table 1).

According to the PAg-ADP% value, we allocated these 219 patients into the clopidogrel-resistant group (157 patients, PAg-ADP% of 77.15 ± 9.60) and control group (62 patients, PAg-ADP% of 44.56 ± 9.73). In the clopidogrel-resistant group, PAg-AA% ($P < 0.001$), white blood cells (WBC, $P = 0.032$), and lymphocytes ($P = 0.030$) showed higher concentrations but platelet count ($P < 0.001$) and D-dimers ($P = 0.023$) showed lower concentrations than the control group (Table 2). In our studies, 124 of 219 patients received the CYP2C19 genotype test. We defined CYP2C19*2 and CYP2C19*3 as CYP2C19 loss-of-function alleles (LoFA), indicating possible clopidogrel resistance.⁸ Upon comparing the PAg-ADP% value, 64.29% (36/56) CYP2C19 LoFA noncarriers (CYP2C19 wild-type homozygotes, *1/*1) and 69.11% (47/68)

Table 1. Main characteristics of patients in the control group and aspirin-resistant group.

Variables	Control group	Aspirin resistant group	P value
Gender (male/female)	(90/28)	(76/25)	0.492
Age (years)	60.92 ± 12.68	61.53 ± 11.87	0.812
Hypertension	50 (42.37%)	49 (48.51%)	0.148
Hyperlipidemia	4 (3.93%)	4 (3.96%)	0.550
Diabetes	32 (27.12%)	32 (31.68%)	0.055
Stroke	9 (7.63%)	8 (7.92%)	0.368
Laboratory data			
PAg-ADP (%)	57.64 ± 1.91	79.94 ± 10.41	<0.001
MAT-AA (s)	451.17 ± 46.29	447.08 ± 43.81	0.125
MAT-ADP (s)	476.07 ± 50.96	473.63 ± 53.13	0.532
PLT ($10^9/L$)	183.16 ± 56.86	156.68 ± 59.77	<0.001
WBC ($10^9/L$)	7.67 ± 2.50	7.93 ± 2.82	0.639
L ($10^9/L$)	1.52 ± 0.69	1.54 ± 0.64	0.816
M ($10^9/L$)	0.57 ± 0.21	0.63 ± 0.32	0.451
RBC ($10^{12}/L$)	4.20 ± 0.61	4.23 ± 0.62	0.670
Hb (g/L)	131.68 ± 19.32	134.03 ± 17.70	0.352
MCV (fL)	92.12 ± 4.88	92.35 ± 4.92	0.719
RDW-CV (%)	0.13 ± 10.01	0.13 ± 0.01	0.978
MPV (fL)	10.47 ± 1.34	10.58 ± 1.30	0.586
PDW (fL)	15.92 ± 11.30	15.59 ± 2.39	0.092
D-D (mg/L)	0.56 ± 0.62	0.80 ± 1.29	0.961
HDL (mmol/L)	3.00 ± 12.50	0.99 ± 0.28	0.542
LDL (mmol/L)	2.20 ± 0.89	2.32 ± 0.88	0.278
TG (mmol/L)	1.58 ± 1.00	1.56 ± 0.72	0.385
TC (mmol/L)	3.87 ± 1.24	4.07 ± 1.25	0.227
HbA1c (%)	6.56 ± 1.38	6.61 ± 1.96	0.623
UA ($\mu\text{mol/L}$)	332.39 ± 107.06	337.88 ± 102.75	0.702
E ($10^9/L$)	0.2 ± 0.11	0.12 ± 0.13	0.736
B ($10^9/L$)	0.03 ± 0.02	0.03 ± 0.02	0.725

PAg-ADP, platelet aggregation rate with adenosine diphosphate; MAT, maximum aggregation time; PLT, platelet; WBC, white blood cells; L, lymphocyte; M, monocyte; RBC, red blood count; Hb, hemoglobin; MCV, mean corpuscular volume; RDW-CV, red blood cell distribution width; MPV, mean platelet volume; PDW, platelet distribution width; D-D, dimers; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglyceride; TC, Cholesterol; UA, uric acid; E, eosinophil; B, basophils.

Table 2. Main characteristics of patients in control group and clopidogrel-resistant group.

Variables	Control group	Clopidogrel resistant group	P value
Gender (male/female)	(45/17)	(121/36)	0.263
Age (years)	60.50 ± 13.43	61.48 ± 11.76	0.766
Hypertension	29 (46.77%)	70 (44.59%)	0.443
Hyperlipidemia	1 (1.61%)	3 (1.91%)	0.682
Diabetes	15 (24.19%)	51 (32.48)	0.149
Stroke	5 (8.06%)	12 (7.64%)	0.556
Laboratory data			
PAg-AA (%)	37.29 ± 12.34	61.13 ± 16.13	<0.001
MAT-AA (s)	455.88 ± 42.20	446.71 ± 46.14	0.186
MAT-ADP (s)	472.34 ± 49.51	475.75 ± 53.63	0.783
PLT (10 ⁹ /L)	196.82 ± 53.70	160.83 ± 58.51	<0.001
WBC (10 ⁹ /L)	7.40 ± 2.93	7.94 ± 2.50	0.032
L (10 ⁹ /L)	1.42 ± 0.72	1.58 ± 0.63	0.030
M (10 ⁹ /L)	0.56 ± 0.21	0.61 ± 0.28	0.338
RBC (10 ¹² /L)	4.11 ± 0.68	4.25 ± 0.58	0.319
Hb (g/L)	128.42 ± 20.96	134.48 ± 17.19	0.129
MCV (fL)	92.39 ± 5.70	92.16 ± 4.51	0.761
RDW-CV (%)	0.13 ± 0.01	0.13 ± 0.01	0.304
MPV (fL)	10.44 ± 1.28	10.56 ± 1.33	0.563
PDW (fL)	16.51 ± 15.29	15.48 ± 2.48	0.051
D-D (mg/L)	0.69 ± 0.71	0.64 ± 1.04	0.023
HDL (mmol/L)	3.50 ± 13.91	1.53 ± 6.49	0.979
LDL (mmol/L)	2.23 ± 1.12	2.26 ± 0.76	0.138
TG (mmol/L)	1.46 ± 0.76	1.62 ± 0.92	0.142
TC (mmol/L)	3.87 ± 1.47	4.00 ± 1.14	0.153
HbA1c (%)	6.36 ± 1.62	6.68 ± 1.67	0.117
UA (μmol/L)	322.56 ± 97.43	339.87 ± 107.00	0.273
E (10 ⁹ /L)	0.10 ± 0.10	0.13 ± 0.12	0.111
B (10 ⁹ /L)	0.03 ± 0.03	0.03 ± 0.02	0.098

PAg-AA, platelet aggregation rate with arachidonic acid

CYP2C19 LoFA carriers (38 patients were CYP2C19*3 or *2 heterozygote, 9 patients were CYP2C19*3/*3 or *2/*3) showed clopidogrel resistance.

In addition, 98 of 219 patients were dual antiplatelet drug resistant with PAg-AA% of 71.11% ± 10.42% and PAg-ADP% of 80.97% ± 8.65%; they showed significantly lower MAT-AA ($P=0.047$), platelet count ($P < 0.001$), and platelet distribution width ($P=0.039$, Table 3) than the control group.

The Risk Factor Assessment of Aspirin and Clopidogrel Resistance

To assess the risk factors of aspirin resistance, the association between clinical characteristics and aspirin resistance was analyzed by logistic regression analysis. The univariate analysis showed that PAg-ADP% (OR: 1.137; 95% confidence interval [CI]: 1.099-1.176), platelet count (OR: 0.992; 95% CI: 0.987-0.997), and monocyte count (OR: 2.460; 95% CI: 0.875-6.920) were associated with aspirin resistance ($P < 0.1$, Table 4). Multivariate analysis of PAg-ADP%, platelet count, monocyte count, age, sex, hypertension, hyperlipidemia, diabetes, and stroke showed that PAg-ADP% (OR: 1.141; 95% CI: 1.099-1.184) to be significantly associated with aspirin resistance (Table 5).

The univariate analysis between clopidogrel resistance and clinical characteristic showed that PAg-AA% (OR: 1.124; 95% CI: 1.086-1.163), platelet count (OR: 0.990; 95% CI: 0.984-0.995), Hb (OR: 1.018; 95% CI: 1.002-1.034), and eosinophil count (OR: 12.490; 95% CI: 0.681-229.241) were associated with clopidogrel resistance ($P < 0.1$, Table 6). Taken together with age, sex, and hypertension, hyperlipidemia, diabetes and stroke into multivariate analysis, PAg-AA% (OR: 1.135; 95% CI: 1.091-1.182), platelet count (OR: 0.986; 95% CI: 0.977-0.995), and Hb (OR: 1.026; 95% CI: 1.002-1.050) were identified as the potential risk factors of clopidogrel resistance (Table 7).

In the dual antiplatelet drug-resistant group, platelet count (OR: 0.991; 95% CI: 0.986-0.996) and monocyte count (OR: 2.460; 95% CI: 0.875-6.920) were found to be associated with dual antiplatelet drug resistance according to univariate analysis ($P < 0.1$, Table 8). Multivariate analysis of platelet count, monocyte count, age, sex, hypertension, hyperlipidemia, diabetes, and stroke showed that platelet and monocyte counts were the potential risk factors of dual antiplatelet drug resistance (Table 9).

Discussion

As the standard treatment of acute myocardial infarction, DAPT is widely used after PCI. Aspirin exerts its antiplatelet function

Table 3. Main characteristics of patients in control group and dual antiplatelet drug -resistant group.

Variables	Control group	Double resistant group	P value
Gender (male/female)	(91/30)	(75/23)	0.261
Age (years)	61.00 ± 12.55	61.46 ± 12.01	0.890
Hypertension	51 (42.15%)	49 (50%)	0.368
Hyperlipidemia	4 (3.31%)	4 (4.08%)	0.368
Diabetes	35 (28.93%)	31 (31.63%)	0.261
Stroke	9 (7.44%)	8 (8.16%)	0.261
Laboratory data			
MAT-AA (s)	452.51 ± 45.94	444.98 ± 44.02	0.047
MAT-ADP (s)	474.85 ± 51.51	474.80 ± 54.00	0.748
PLT (10 ⁹ /L)	184.11 ± 56.81	154.73 ± 59.18	<0.001
WBC (10 ⁹ /L)	7.68 ± 2.56	7.93 ± 2.76	0.556
L (10 ⁹ /L)	1.53 ± 0.70	1.52 ± 0.62	0.947
M (10 ⁹ /L)	0.57 ± 0.21	0.63 ± 0.32	0.367
RBC (10 ¹² /L)	4.20 ± 0.61	4.23 ± 0.62	0.653
Hb (g/L)	131.79 ± 19.126	133.96 ± 17.93	0.699
MCV (fL)	92.26 ± 5.02	92.19 ± 4.75	0.923
RDW-CV (%)	0.13 ± 0.01	0.13 ± 0.01	0.714
MPV (fL)	10.49 ± 1.33	10.57 ± 1.31	0.644
PDW (fL)	15.84 ± 11.17	15.68 ± 2.36	0.039
D-D (mg/L)	0.56 ± 0.62	0.80 ± 1.30	0.883
HDL (mmol/L)	2.95 ± 12.35	0.98 ± 0.28	0.416
LDL (mmol/L)	2.25 ± 0.96	2.25 ± 0.78	0.618
TG (mmol/L)	1.59 ± 0.98	1.56 ± 0.73	0.525
TC (mmol/L)	3.94 ± 1.34	3.98 ± 1.18	0.503
HbA1c (%)	6.56 ± 1.38	6.61 ± 1.98	0.667
UA (μmol/L)	332.17 ± 106.30	338.33 ± 103.56	0.668
E (10 ⁹ /L)	0.12 ± 0.11	0.12 ± 0.12	0.872
B (10 ⁹ /L)	0.03 ± 0.02	0.03 ± 0.02	0.684

by acetylating the platelet cyclooxygenase 2 (COX-2) at serine 516 and COX-1 at 529, which converts AA into thromboxane A₂. Although it is an important prescribed drug for cardiovascular and cerebrovascular diseases, resistance to its effects limits its efficiency.^{9,10} There are reported 5%–45% patients with aspirin resistance;¹¹ in addition, the following factors contribute to aspirin resistance: the COX-1 polymorphisms of C50 T, -A842G, and A1676G; the COX-2 polymorphism of -765; single nucleotide polymorphisms, such as the rs1126643 of the GPIa and the rs5918 of GPIIIa,^{12,13} diseases like inflammation (mediated by oxidative stress-induced 8-isoprostaglandin F_{2α}, white blood cell count, monocyte count, and macrophages),^{13,14} diabetes (related to waist circumference, HOMA-IR, QUICKI, and leptin),¹⁵ obesity,^{16,17} patient compliance and miRNAs.^{18,19} In this study, we compared the control group and aspirin-resistant group and found that PAg-ADP% and platelet counts were significantly different. Moreover, PAg-ADP% and PAg-AA% had a linear correlation, and clopidogrel resistance could increase the risk of developing aspirin resistance. The ADP receptor P2Y₁ (P2RY1) seems to be the bridge between aspirin resistance and clopidogrel resistance. The A1622G mutation of the P2RY1 gene reportedly increased the risk of aspirin resistance in major adverse cardiovascular and cerebrovascular events, and the A236G variant impacted clopidogrel resistance in cats with hypertrophic cardiomyopathy.^{20,21}

Platelet count has been reportedly associated with aspirin response before. Contrary to our findings, it has been reported that patients with aspirin resistance have higher platelet count and that other concomitant common cardiovascular medications, such as angiotensin receptor blocker and calcium channel blocker, may contribute to the higher platelet count in aspirin-resistant patients.²²

Although diabetes, obesity, and high cholesterol have been reported to be associated with aspirin resistance before, we did not find any significant difference between aspirin-resistant and control groups; this could be attributed to clopidogrel treatment or PCI affecting aspirin resistance.

Clopidogrel is the other drug of DAPT; it exerts its function by inhibiting the purinergic receptor P2Y₁₂ and ADP-induced platelet aggregation. As a prodrug, clopidogrel needs cytochrome P450 (CYP) enzymes to transform it into its active form, and therefore, the variation of CYP enzymes will influence clopidogrel efficacy. CYP2C19*2, CYP2C19*3, and CYP2C19*17 have been proved to contribute to clopidogrel resistance development.^{8,23} A meta-analysis encompassing 12 studies showed that CYP2C19*2 was more strongly associated with clopidogrel resistance, particularly in Asian population.²⁴ As CYP2C19*17 has lesser prevalence in general population,²⁵ we herein compared the results among CYP2C19 genotypes (CYP2C19*1, CYP2C19*2, and CYP2C19*3) in terms of PAg-ADP% in 124 patients and found that the CYP2C19 LoFA noncarrier

Table 4. The univariate logistic regression analysis of aspirin resistance risk factors.

Variables	OR	95%CI	P value
Gender (male/female)	0.918	0.488-1.728	0.792
Age (years)	1.003	0.981-1.025	0.783
Hypertension	1.318	0.771-2.252	0.313
Hyperlipidemia	1.245	0.303-5.110	0.761
Diabetes	1.137	0.637-2.029	0.664
Stroke	1.106	0.410-2.983	0.832
PAG-AA (%)	2.892	1.592-5.253	<0.001
PAG-ADP (%)	1.175	1.124-1.228	<0.001
MAT-AA (s)	0.998	0.992-1.004	0.513
MAT-ADP (s)	0.999	0.994-1.004	0.734
PLT ($10^9/L$)	0.991	0.986-0.996	<0.001
WBC ($10^9/L$)	1.036	0.936-1.146	0.494
L ($10^9/L$)	0.986	0.660-1.475	0.946
M ($10^9/L$)	2.460	0.875-6.920	0.088
RBC ($10^{12}/L$)	1.106	0.715-1.709	0.652
Hb (g/L)	1.006	0.992-1.021	0.391
MCV (fL)	0.997	0.944-1.053	0.923
RDW-CV (%)	0.054	0.000-5.501E9	0.822
MPV (fL)	1.049	0.857-1.284	0.643
PDW (fL)	0.998	0.966-1.030	0.886
D-D (mg/l)	1.315	0.929-1.863	0.123
HDL (mmol/L)	0.986	0.423-1.899	0.775
LDL (mmol/L)	1.002	0.737-1.363	0.989
TG (mmol/L)	0.964	0.706-1.315	0.816
TC (mmol/L)	1.027	0.826-1.278	0.811
HbA1c (%)	1.016	0.863-1.195	0.851
UA ($\mu\text{mol/L}$)	1.001	0.998-1.003	0.666
E ($10^9/L$)	1.265	0.130-12.310	0.839
B ($10^9/L$)	4.255	0.000-433454.445	0.806

Table 5. The multivariate logistic regression analysis of aspirin resistance risk factors.

Variables	OR	95%CI	P value
Gender	0.476	0.173-1.311	0.151
Age	0.993	0.959-1.029	0.715
Hypertension	1.315	0.578-2.989	0.513
Hyperlipidemia	1.836	0.281-11.980	0.526
Diabetes	0.788	0.346-1.796	0.570
Stroke	0.528	0.136-2.053	0.356
PLT	1.000	0.992-1.008	0.987
PAG-ADP	1.141	1.099-1.184	<0.001
M	2.841	0.675-11.954	0.154

group showed lower clopidogrel resistance (PAG-ADP% > 55%) than the CYP2C19 LoFA carrier group. This verified the importance of the CYP2C19 genotype test and platelet function test; conversely, 45.71% patients still showed clopidogrel resistance as per PAG-ADP% among CYP2C19 LoFA noncarriers, which may guide improper clopidogrel dosage. In patients who are insensitive to active clopidogrel, the inadequate patient number and the definition of clopidogrel resistance may affect the results. Besides exhibiting differences in the expression of CYP enzymes, we found that the clopidogrel-resistant group showed higher PAG-AA%, higher lymphocyte and WBC

counts, and lower platelet count and D-dimers. According to multivariate analyses, PAG-AA%, platelet count, and hemoglobin were independent predictors for clopidogrel resistance.

As we have shown that PAG-ADP% and PAG-AA% have a linear correlation and P2RY1 may be the bridge, it is not surprising that PAG-AA% is higher in the clopidogrel-resistant group and that aspirin resistance was an independent variable associated with clopidogrel resistance after PCI.²⁶ Several studies have reported on the relationship between inflammation and clopidogrel resistance: A review showed that inflammation could induce thrombosis, and systemic inflammation could cause aspirin and clopidogrel resistance;²⁷ two studies showed that higher lymphocyte counts were correlated with increased clopidogrel resistance.^{28,29} Moreover, the higher interleukin (IL)-10, IL-4, C-reactive protein, IL-6, P-section, CD40L, and MIP-1 β and lower I-TAC were associated with increased clopidogrel resistance.^{30,31} Regarding the concentration of CD40L, there was another different result from 378 clopidogrel-naive patients: it showed that CD40L concentration did not significantly differ between clopidogrel-resistant and control groups.²⁹ In contrast, clopidogrel also affected the expression of inflammation factors such as CD40L and P-section and inhibited platelet antigen formation.²⁷

Platelet counts showed important differences among clopidogrel-resistant patients, which is in agreement with our

Table 6. The univariate logistic regression analysis of clopidogrel resistance risk factors.

Variables	OR	95%CI	P value
Gender	1.317	0.672-2.580	0.423
Ages	1.007	0.983-1.031	0.593
Hypertension	0.916	0.508-1.651	0.769
Hyperlipidemia	1.192	0.234-6.072	0.832
Diabetes	1.508	0.771-2.949	0.230
Stroke	0.943	0.318-2.799	0.916
PAg-AA (%)	1.124	1.086-1.163	<0.001
MAT-AA (s)	0.995	0.988-1.002	0.183
MAT-ADP (s)	1.001	0.996-1.007	0.669
PLT ($10^9/L$)	0.990	0.984-0.995	<0.001
WBC ($10^9/L$)	1.086	0.965-1.223	0.172
L ($10^9/L$)	1.467	0.915-2.352	0.112
M ($10^9/L$)	2.052	0.618-6.809	0.240
RBC ($10^{12}/L$)	1.437	0.887-2.329	0.141
Hb (g/L)	1.018	1.002-1.034	0.032
MCV (fL)	0.991	0.933-1.052	0.760
RDW-CV (%)	<0.001	0.000-82.254	0.101
MPV (fL)	1.069	0.853-1.399	0.562
PDW (fL)	0.987	0.955-1.021	0.452
D-D (mg/l)	0.955	0.685-1.332	0.766
HDL (mmol/L)	0.980	0.951-1.011	0.205
LDL (mmol/L)	1.050	0.745-1.479	0.780
TG (mmol/L)	1.286	0.861-1.923	0.219
TC (mmol/L)	1.091	0.852-1.397	0.490
HbA1c (%)	1.135	0.933-1.380	0.204
UA ($\mu\text{mol/L}$)	1.002	0.999-1.004	0.273
E ($10^9/L$)	12.490	0.681-229.241	0.089
B ($10^9/L$)	25.927	0-2.039E7	0.638

Table 7. The multivariate logistic regression analysis of clopidogrel resistance risk factors.

Variables	OR	95%CI	P value
Gender	0.396	0.129-1.209	0.104
Age	0.977	0.936-1.019	0.281
Hypertension	0.621	0.245-1.573	0.315
Hyperlipidemia	0.983	0.114-8.448	0.988
Diabetes	2.037	0.764-5.431	0.155
Stroke	0.997	0.109-5.894	0.998
PAg-AA	1.135	1.091-1.182	<0.001
PLT	0.986	0.977-0.995	0.002
Hb	1.026	1.002-1.050	0.03
E	23.181	0.326-1648.682	0.49

findings; lower platelet counts were found to be associated with increased clopidogrel resistance in Han Chinese population.³² However, Uzun et al. verified that a higher platelet count is an independent factor of clopidogrel resistance in 207 PCI patients from Turkey;²⁶ Marginean et al. reported similar findings in a study conducted in Romania. Whether or not racial differences contributed to the difference remains unknown.²⁸

We found hemoglobin as an independent factor for clopidogrel resistance. We noted lower concentrations of hemoglobin in the clopidogrel-resistant group after PCI, and hemoglobin being <13.9, BMI being >28 kg/m², and diabetes

Table 8. The univariate logistic regression analysis of dual antiplatelet drug resistance risk factors.

Variables	OR	95%CI	P value
Gender	0.918	0.488-1.728	0.792
Ages	1.003	0.981-1.025	0.783
Hypertension	1.318	0.771-2.252	0.313
Hyperlipidemia	1.245	0.303-5.110	0.761
Diabetes	1.137	0.637-2.029	0.664
Stroke	1.106	0.410-2.983	0.842
MAT-AA (s)	0.996	0.990-1.002	0.234
MAT-ADP (s)	1.000	0.995-1.005	0.995
PLT ($10^9/L$)	0.991	0.986-0.996	<0.001
WBC ($10^9/L$)	1.036	0.936-1.146	0.494
L ($10^9/L$)	0.986	0.660-1.475	0.946
M ($10^9/L$)	2.460	0.875-6.920	0.088
RBC ($10^{12}/L$)	1.106	0.715-1.709	0.652
Hb (g/L)	1.006	0.992-1.021	0.391
MCV (fL)	0.997	0.944-1.053	0.923
RDW-CV (%)	0.054	0.000-5501E9	0.822
MPV (fL)	1.049	0.875-1.284	0.643
PDW (fL)	0.998	0.966-1.030	0.886
D-D (mg/l)	1.315	0.929-1.863	0.123
HDL (mmol/L)	0.896	0.423-1.899	0.775
LDL (mmol/L)	1.002	0.737-1.363	0.989
TG (mmol/L)	0.964	0.706-1.315	0.816
TC (mmol/L)	1.027	0.826-1.278	0.811
HbA1c (%)	1.116	0.863-1.195	0.851
UA ($\mu\text{mol/L}$)	1.001	0.998-1.003	0.666
E ($10^9/L$)	1.265	0.130-12.310	0.839
B ($10^9/L$)	4.255	0.000-433454.445	0.806

Table 9. The multivariate logistic regression analysis of dual antiplatelet drug resistance risk factors.

Variables	OR	95%CI	P value
Gender	1.489	0.715-3.101	0.287
Age	0.980	0.954-1.006	0.132
Hypertension	1.488	0.804-2.753	0.206
Hyperlipidemia	1.424	0.315-6.434	0.646
Diabetes	0.852	0.451-1.608	0.621
Stroke	0.918	0.323-2.608	0.872
PLT	0.989	0.983-0.994	0.001
M	4.177	1.267-13.772	0.019

could predict clopidogrel-resistance.^{26,33} Elevated levels of D-dimers were proven as an independent hemostatic predictor of thrombotic events in 123 patients as D-dimers were directly associated with the activity of t-PA,³⁴ and antiplatelet agents could not influence D-dimer levels.²⁶ In our study, we found D-dimers to be associated with clopidogrel resistance; however, the underlying mechanism remains unclear.

Dual antiplatelet drug-resistant patients showed lower maximum aggregation time (the time needed for maximum PAg), platelet count, and platelet distribution width during our study. Platelet and monocyte counts were independent risk factors of dual antiplatelet drug resistance. In addition, a

study found that an increased immature platelet count weakens dual antiplatelet drug resistance, and changing clopidogrel to ticagrelor could increase the immature platelet count.³⁵ Since little research has focused on monocyte count being an independent factor for dual antiplatelet drug resistance, this needs more verification and exploration.

The most valuable finding of the present study was aspirin resistance and clopidogrel resistance showing a linear correlation. All of these findings confirm that DAPT failure can be very dangerous. Therefore, it is instrumental to monitor AA- and ADP-induced platelet aggregation for patients treated with DAPT. When aspirin or clopidogrel resistance was identified, increasing the aspirin dose by 300 mg,³⁶ doubling the clopidogrel dose,²⁸ while maintaining concomitant administration of other drugs, such as GPI/IIIa inhibitors and angiotensin receptor blocker, could reduce the risk of re-infarction. Moreover, monitoring the PAg-AA% and PAg-ADP% is significant for individualized medicine not only for PCI patients but also for patients treated with antiplatelet therapy. When the values of PAg-AA% and PAg-ADP% are suggestive of the occurrence of resistance, adding drug dosage or changing the drug should be considered.

Limitation

Even though we found several risk factors associated with aspirin and clopidogrel resistance and discovered a relationship between aspirin resistance and clopidogrel, the detailed mechanism behind inflammation influencing drug resistance remains unknown. Moreover, the guiding functions of platelet count and lymphocyte are minimal as their values are still within the standard range. For the study itself, we used only one method to test the aggregation, and the inadequate number of patients may influence the reliability of the results. Furthermore, the results only reflected patients having undergone aspirin and clopidogrel therapies. In addition, patients with other diseases besides hypertension, hyperlipidemia, diabetes, and stroke were not included. Habits like alcohol intake and smoking can also affect the results but were not accounted for in this study. In addition, a healthy control group to verify the effectiveness of drugs and clinical follow-up was not included.

Conclusion

In conclusion, PAg-ADP% is the risk factor for aspirin resistance; PAg-AA%, hemoglobin, and platelet count are the risk factors of clopidogrel resistance. Lower platelet counts and platelet distribution width were associated with an increased risk of dual antiplatelet drug resistance. When patients are treated with DAPT, more attention should be paid to check for concurrent aspirin and clopidogrel resistance.

Author Contributions

Lijie Zhang: Conceptualization, methodology, writing the original draft, and funding acquisition. **Ying Lv:** Conceptualization and data curation; **Jianyu Dong:** Conceptualization, methodology, and

writing the original draft; **Nana Wang:** Methodology, software, and validation; **Zhan Zhan:** Supervision and validation; **Yuan Zhao:** Data curation and supervision; **Shanshan Jiang:** Conceptualization, writing (review and editing), supervision, and funding acquisition.

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Ethics Approval and Informed Consent

Ethical approval for this paper was obtained from Ethics Committee of Shaanxi Provincial People's Hospital and Ethics Committee of Xi'an Jiaotong University (2018-379). The need for patients' informed consent for publishing their information was waived due to the retrospective design of the study.


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ORCID iD

Shanshan Jiang  <https://orcid.org/0000-0002-7841-9448>

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