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Efficacy of Monitoring Platelet Function by an Automated PL-12 Analyzer During the Treatment of Acute Cerebral Infarction With Antiplatelet Medicine

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Abstract

Numerous methods can be used to investigate the function of platelets; however, technical issues limit tends to limit the applicability of such methods in the clinic. **Methods:** All participants were administered with oral aspirin (100 mg/d) for 7 days. Blood samples were then collected and platelet function evaluated by an automated PL-12 analyzer, TEG, and the platelet count drop method. We found that platelet counts determined by the traditional platelet drop method were significantly lower in the PL-12 sensitive group and significantly higher in the PL-12 insensitive group ($P < 0.05$). Furthermore, MAR measured by PL-12 was positively correlated with the MA values determined by TEG and the platelet drop method ($r = 0.322$, $r = 0.036$, respectively, $P < 0.05$), More importantly, the PL-12 analyzer showed the largest AUC (0.748) with a sensitivity of 87.4% and a specificity of 57.4%, indicating PL-12 analyzer using in platelet aggregation evaluation of ACI patients more credibly and accuracy. Additionally, genetic analysis showed that the polymorphic of A-allele in the PEAR1 (rs12041331) gene was significantly increased in the PL-12 sensitive group rather than in the PL-12 insensitive group ($P < 0.05$), suggesting the predictive value of PL-12 analyzer for the prognosis of ACI patients was superior to the other methods tested herein. Our analyses demonstrate that PL-12 analysis offer a new superior technology for monitoring antiplatelet drug efficacy and for clinical prognosis of ACI patients, which has the advantages of simplicity, speed, and automation in platelet aggregation measurement.

Keywords

acute cerebral infarction, aspirin, thrombelastography, PL-12 analyzer

Introduction

Cerebrovascular disease is 1 of the top 3 diseases globally, is associated with high incidence and mortality rates, disability rates, and recurrence rates.[1,2] Acute cerebral infarction (ACI), also known as acute ischemic stroke (AIS), has an incidence rate of 50% -80% and a disability rate of 50% to 70%, and has become the most common disorder related to cerebrovascular disease.[3,4] Anti-platelet aggregation therapy has been confirmed as an effective treatment for AIS not only due to its effects with regard to acute treatment, but also for its effects on intervention and early secondary prevention.[5,6] Aspirin or clopidogrel monotherapy currently represents the first choice for antiplatelet function treatment; indeed, aspirin is also regarded as the gold standard for preventing cardiovascular and cerebrovascular events, with better economic benefits from other medicine therapy for antiplatelet function.[7] Despite receiving aspirin in strict accordance with current clinical guidelines, many patients with AIS continue to experience recurrent vascular events due to reduced platelet sensitivity. Clinical investigations have shown that approximately 3%–85% of AIS patients taking aspirin, and between 4%–30% of AIS patients taking clopidogrel, are at risk of ischemic stroke and show no improvements in terms of clinical outcome[8,9]; this condition can be interpreted as 'aspirin/clopidogrel resistance'.[10-12] Consequently, it is vital that we develop appropriate ways to measure platelet function if we are to monitor the

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efficacy of aspirin and thus be able to manage ACI therapy and evaluate patient prognosis in an accurate and efficient manner.

A number of methods have been used in the clinic in order to determine platelet function, including light transmission aggregation (LTA), 'VerifyNow', and thromboelastography (TEG). However, there is still a lack of unified evidence to fully support any of these techniques with regard to accuracy and reliability. Although each of these methods has its own advantages, there are still a number of technical limitations that need to be overcome.[13,14] LTA is currently considered as the gold standard and is most commonly used for the identification and diagnosis of platelet dysfunction; however, this technique is also labor intensive and time consuming, and is not as standardized as other tests.[15] TEG is a typical method for monitoring the process of hemostasis, from initiating coagulation systems to the dissolution of blood clots; this method is a useful way of showing interactions between different components in the whole blood, including platelets and clotting systems. Furthermore, by comprehensively analyzing different parameters TEG can facilitate the qualitative diagnosis of coagulation function.[16] However, TEG is relatively insensitive to all aspects of platelet function. Certain genomic polymorphisms have also been associated with the risk of bleeding events after antiplatelet therapy,[17,18] although the detection of such polymorphisms is also associated with several limitations, including the fact that patients willing to take the drug resistance gene test in clinic are often insufficient, and the conclusions between the antiplatelet aggravation and the drug-resistant gene are often controversial.

The PL-12 is a novel automatic platelet function analyzer and regarded as an upgrade version of PL-11, which was developed from the principles of the platelet count drop method.[19] This method detects single platelet changes before and after supplementation with agonists into whole citrated blood using a standard electrical impedance cell counter. According to previous research, the PL-11 impedance method is a reliable and accurate method for measuring platelet aggregation function and shows good levels of correlation with VerifyNow, vasodilator stimulated phosphoprotein (VASP) assay, and moderate levels of correlations with both LTA and TEG.[19] However, as an upgraded version of PL-11, the PL-12 analyzer has yet to be characterized with respect to platelet aggregation function. Therefore, we aimed to investigate the role of the PL-12 analyzer to monitor short-term responses to aspirin in patients with ACI in comparison with 2 other commonly used and well-established platelet function assays (TEG and platelet counting) and a gene detection assay for drug resistance. In addition, we compared the predictive value of the PL-12 analyzer with various detection methods for adverse cardiovascular events in order to provide further guidance for developing precise treatments for ACI.

Materials and Methods

Study Subjects

A total of 370 patients with ACI were recruited from The First Affiliated Hospital of Xiamen University in China between September 2017 and September 2019. There were 277 males and 93 females, aged from 32 to 85 years, with mean age of 66.42 $\pm$ 10.61 years. A range of general clinical data were collected on admission, including age, gender, smoking, hypertension, macrovascular disease, and National Institute of Health Stroke Scale (NIHSS) score.

The inclusion criteria were as follows: 1) patients aged from 18 to 85 years old who required antiplatelet therapy within 48 hours after onset; 2) patients with ACI, as diagnosed by magnetic resonance image (MRI) or computer tomography (CT), in full conformity with the diagnostic criteria for ACI; and 3) patients experiencing their first episode of ACI without taking any form of antiplatelet drug previously, and who took aspirin regularly for more than 7 days after admission. The exclusion criteria were as follows: 1) cardiogenic cerebral infarction, including atrial fibrillation; 2) a history of intracranial bleeding or subarachnoid hemorrhage; 3) patients with contraindications to antiplatelet therapy; 4) patients with severe heart, liver, and kidney dysfunction; and 5) patients with coagulopathy and bleeding tendency. All participants provided informed consent prior to the participating in the study. The study was carried out in accordance with the tenets of the Declaration of Helsinki and was supported by the Ethics Committee of The First Affiliated Hospital of Xiamen University.

Neurological Dysfunction

Neural function was assessed by the NHISS[20] before aspirin treatment and 7 days after treatment began. This scale features 0–28 points; a high score indicates a poorer recovery of neurological functions, and vice versa. The modified Rankin Scale (mRS) was used to evaluate the ability of our patients to carry our activities related to daily life. mRS scores range from 0 to 6; a score of 0 represents no symptoms while a score of 6 indicates death. The higher the score, the more severe the disability and the less able the patient is with regard to normal daily activities.

Treatment Protocols

Following the collection of clinical data, all of the enrolled patients were asked to take oral aspirin (enteric-coated tablets; Bayer S. p. A., J20130078; 100 mg) once daily for 7 days. During this time period, participants also received treatment to provide nutrition for their nervous system, to improve circulation, to control lipid levels, and other forms of treatment. All subjects were administered with a single form of antiplatelet medicine throughout the trial; this would not be replaced unless hemorrhagic or ischemic events were encountered by the patient, or unless the patient withdrew from the experiment.

Blood Samples

After 7 days of aspirin treatment, we acquired blood samples from the antecubital vein of all patients so that we could monitor platelet function. Five tubes of blood were collected from

each of the patients treated with aspirin, which containing 2 ml blood in each tube: 2 tubes were pre-treated with 3.2% sodium citrate (Becton-Dickinson, USA) for PL-12 and TEG tests. A third tube was pre-treated with heparin (Becton-Dickinson) and used for the TEG test. The other 2 tubes were pre-treated with ethylenediaminetetraacetic acid and used for genotyping and platelet counting. During the collection of blood samples, we did not use a tourniquet; this was to avoid the activation of platelets. We also discarded the first 2 ml of blood. In addition, the 5 tubes were gently inverted 3 times in order to ensure that the contents were completely mixed with anticoagulant. One tube of blood was preserved at −80°C for genotyping; the rest were stored at room temperature to await platelet function tests; these tests were carried out within 2 hours of the blood samples being acquired.

Use of the PL-12 Analyzer to Measure Platelet Function

The PL-12 platelet function analyzer (SINNOWA Medical Science & Technology Co., Nanjing, China) is a new point-of-care apparatus for analyzing platelet function and is based upon an automated impedance technique. The PL-12 analyzer features an automated impedance-based hematology analyzer and agonist kits; these can automatically and continuously count changes in platelet counts in whole blood/sodium citrate before and after the addition of an inducer during a fixed time interval. The protocol is very simple; 500 μL of citrated blood sample was placed into a polycarbonate tube and inserted into the measuring position; the remainder of the procedure is automated. Platelet count was determined twice at the beginning of the procedure to obtain a mean baseline value. Care was taken to ensure that blood samples in the polycarbonate tubes were mixed gently during the whole course of detection and that a short time interval was allocated between each test point to allow for system cleaning. Prior to the third detection time point, an arachidonic acid (AA) inducer (final concentration: 50 μmol/L) was added into the blood sample to activate platelet aggregation. Since aggregation created structures that were too large to be counted as single platelets, the single platelet count dropped as platelet aggregation increases. The PL-12 analyzer counted platelets several times until the lowest level of single platelet count was obtained. The entire procedure was accomplished within 15 min (6 detection time points) and the temperature was maintained at 37°C. The maximal platelet aggregation rate (MAR) ratio was determined for each sample using the following formula:

$$MAR = [(baseline - lowest\ platelet\ count)/baseline] \times 100\%.$$

An MAR < 60% was defined as having a high response to aspirin (the PL-12 sensitivity group), while an MAR ≥ 60% was defined as a low response to aspirin (the PL-12 insensitivity group).

Locomotor Functional Testing

Two blood samples were extracted from each patient, stored in heparin tubes that were pre-treated with 3.2% sodium citrate, and used for the TEG test. Images and indicators from the TEG were both indicative of platelet function and were measured by a TEG YZ5000 elastic instrument (Haemoscope Corporation, Niles, IL, USA); AA was used to induce platelet aggregation. Different anticoagulant venous blood samples and agonists were placed into special cuvettes and the whole blood was activated in a tube with a tension wire that was rotated gently. When the AA agonist was added to the blood, the resistance to the movement of the wire increased coincident with platelet aggregation and the formation of clots. The maximal clot strength generated was referred to as maximum amplitude (MA) and was presented graphically on the TEG trace. In this test, 4 key TEG parameters were generated: R value (coagulation reaction time), K value (coagulation time), Angle (which reflects the rate of formation for blood clots and the function of fibrinogen) and MA (maximum thrombus amplitude). The coagulation index (CI) was also calculated for each of these parameters.

Single Nucleotide Polymorphism (SNP) Selection Criteria

In this study, we aimed to carry out genotyping and SNP analysis to evaluate aspirin responsivity in patients with ACI, as described previously. To do this, we genotyped all 5 known SNPs in platelet glycoprotein (GP) IIb receptors (*GP IIb*, rs5911), heme oxygenase 1 (*HO-1*, rs2071746), prostaglandin-endoperoxide synthase 1 (*PTGS1*, rs10306114), cyclooxygenase 2 (*COX-2*, rs20417) and platelet endothelial aggregation receptor 1 (*PEAR1*, rs12041331). The polymorphism of GP IIb/IIIa receptors, notably in *GP IIb,* is known to exert a critical role in platelet aggregation or aspirin resistance.[21,22] In addition, the polymorphism of *HO-1* (rs2071746) has been confirmed to be involved in the pathophysiological processes underlying arteriosclerotic stroke.[23] A previous study also indicated that the polymorphism of *PTGS1* (rs10306114) played a significant role in increasing the risk of bleeding complications in patients undergoing elective coronary angiography.[24] Furthermore, polymorphisms in *COX-2* (rs20417) have been correlated with subclinical atherosclerosis,[25] most probably due to its alternative effect on the function of COX-2 enzyme and its effect on the synthesis of prostaglandins,[26,27] which is involved in the pathogenesis of arterial thrombosis. The polymorphisms of *PEAR1* (rs12041331) are also known to be closely related to platelet function.[28]

SNP Genotyping

The genotype of all 5 SNPs in *GPIIb* (rs5911), *HO-1* (rs2071746), *PTGS1*(rs10306114), *COX-2* (rs20417, and *PEAR1* (rs12041331) were determined with polymerase chain-reaction-based restriction fragment length polymorphism, as described previously.[29] In brief, genomic DNA was isolated from whole blood using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) in accordance with the manufacturer's instructions. Subsequently, 100 ng of DNA was added to a 30 μL PCR reaction volume; the reaction conditions were as follows: pre-denaturation was carried out at

Table 1. PCR Primers for 7 SNPs in 5 Key Genes: *GPIIb*, *HO-1*, *PTGS1*, *COX-2* and *PEAR1*.

Name	SNP ID	Variant	Forward (5'→3')	Reverse (5'→3')
GP IIb	rs5911	A>C		
HO-1	rs2071746	A>T	TCAGCAGAGGATTCCAGCAGGTG	AGG CAG CGC TGC TCA GAG CAC
PTGS1	rs10306114	A>G	TTCGAGCTGGACTTGGAATG	ACTCATTTTTATCCCCAAGCCATC
COX-2	rs20417	G>C	GCCTTAAGGCATACGTTTTGG	TACTGTTCTCCGTACCTTCAC
PEAR1	rs12041331	G>A	AGGTGAGGGGTTATCCTATGCTA	GGTGGACAAGAGGATCCATTTCT

Abbreviations: *GP IIb*, platelet glycoprotein (GP) IIb receptors; *HO-1*, heme oxygenase 1; *PTGS1*, prostaglandin-endoperoxide synthase 1; *COX-2*, cyclooxygenase 2; *PEAR1*, platelet endothelial aggregation receptor 1.

95°C for 2 minutes followed by denaturation at 95°C for 30 seconds. Then, annealing was carried out at 58°C for 30 s, and extension was carried out at 72°C for 30 s; this was followed by a final extension step at 72°C for 5 min, total 32 cycles.

Genotyping for rs5911 was then performed using the Taqman probe technique. Genotyping for the other SNPs was performed on an ABI PRISM 7900 HT Fast Real-Time instrument and an ABI PRISM 3730 DNA Sequencer (Applied Biosystems, Foster City, CA, USA), respectively. The PCR primers used to amplify the SNPs are shown in Table 1.

Follow-Up

Follow-up data were obtained from a special outpatient clinic, or by telephone contact with the patient by specifically trained research staff, at 1, 3, 6, 9, and 12 months. The main endpoint during the 12 months follow-up period was the recurrence of cerebral infarction, myocardial infarction, bleeding events (such as cerebral hemorrhage, gastrointestinal bleeding or other bleeding events) or all-cause death. Patients all received mRS evaluations 3 months after discharge.

The recurrence of cerebral infarction recurrence was referred to as a sudden functional deterioration in neurological status caused by an ischemic or hemorrhagic event that was verified by NIHSS and neuroimaging (CT or MRI).[30] Bleeding events were judged in accordance with the criteria published by Bleeding Academic Research Consortium (BARC).[31]

Statistical Analysis

Data are presented as means $\pm$ standard deviations and were analyzed by SPSS 21.0 statistical software (SPSS Inc, Chicago, IL, USA). The Kolmogorov–Smirnov test was used to test whether raw data conformed to the normal distribution. The Mann-Whitney U test and Least significant difference analysis was used to test data that were not normally distributed. Comparison between the 2 groups were analyzed by the Chi-squared test and the Student's t-test. The Chi-squared test or Fisher's exact test was used to test whether the distribution of genotypes complied with Hardy-Weinberg equilibrium (HWE). Pearson's correlation coefficient was used to evaluate associations between different methods. Receiver-operating characteristic (ROC) curve analysis was used to define cut off values for each method; we reported the absolute differences for each method along with corresponding 95% confidence intervals. $P < 0.05$ was considered to be statistically significant.

Results

Comparison of Baseline Characteristics Between the Aspirin-Sensitive and Aspirin-Insensitive Group

All of enrolled patients with ACI were treated with antiplatelet medicine (aspirin) for 7 days. A PL-12 analyzer was then used to determine platelet function in each patient; 197 patients with a MAR $< 60\%$ were classified as a PL-12-sensitive group, while 173 patients with a MAR $\geq 60\%$ were classified as a PL-12-insensitive group. We then compared general patient data between the 2 groups. There were no significant differences between the 2 groups in terms of age, body mass index, concomitant disease, biochemical indicators, and neurological assessment on admission ($P > 0.05$). However, we did identify several significant differences between the 2 groups, including gender, smoking status, and drinking status ($P < 0.05$, Table 2).

Next, we compared the efficacy of aspirin in patients by comparing platelet function between the 2 groups. We found that the MAR of platelets in the PL-12-sensitive group was $49.25\% \pm 7.23\%$; in contrast, the MAR of platelets in the PL-12-insensitive group was significantly higher ($67.16\% \pm 5.32\%$; $P < 0.05$). We also compared changes in the NIHSS scores of patients between the 2 groups after taking aspirin for 7 days. We found that compared with the NIHSS scores on admission, the NIHSS scores of both groups had fallen markedly by discharge; the NIHSS score of the PL-12-sensitive group had decreased more than that of the PL-12 insensitive group. We then compared the improvements in NIHSS score between the 2 groups and found that the NIHSS scores in the PL-12-sensitive group were significantly higher than those in the PL-12-insensitive group ($P < 0.05$, Table 3). This finding indicated that platelet therapy (aspirin) significantly reduced the MAR and NIHSS scores in the PL-12-sensitive group.

The Detection of Platelet Function by the Automated PL-12 Analyzer was Consistent With the Platelet Count Drop Method and Thrombelastogram

To confirm the reliability of the novel PL-12 analyzer, we investigated whether the automated detection of platelet

Table 2. A Comparison of Basic Patient Characteristics Between PL-12-Sensitive and PL-12 Insensitive Groups.[a]

	PL-12 sensitive group (n = 197)	PL-12 insensitive group (n = 173)	t/χ2	*P*
Age (years)	67.32 ± 11.25	66.03 ± 10.31	1.144	0.253
Sex (male, %)	151 (76.6%)	92 (53.2%)	85.241	<0.001
Body mass index (kg/m^2)	24.75 ± 1.21	24.49 ± 1.34	1.961	0.051
Smoker (%)	97 (49.2%)	55 (31.8%)	11.583	<0.001
Drinker (%)	58 (29.4%)	23 (13.3%)	14.044	<0.001
Hypertension (%)	144 (73.1%)	126 (72.8%)	0.003	0.954
Diabetes (%)	67 (34.0%)	63 (36.4%)	0.234	0.629
Peripheral vascular disease (%)	28 (14.21%)	22 (12.71%)	0.176	0.674
Cholesterol	4.68 ± 1.32	4.59 ± 1.27	0.666	0.506
TG (mmol/L)	1.53 ± 1.15	1.42 ± 1.21	0.896	0.371
HDL (mg/dL)	1.19 ± 0.71	1.26 ± 0.79	0.898	0.370
LDL (mg/dL)	2.81 ± 1.16	2.69 ± 1.09	1.021	0.308
Admission NIHSS	4.67 ± 2.73	4.69 ± 3.02	0.067	0.947
Admission mRs	1.83 ±0.62	1.98 ± 0.61	2.340	0.198

Abbreviations: TG, triglyceride; HDL, high-density lipoprotein; LD, high-density lipoprotein.
[a]Values are presented as mean ± standard deviation or n (%).

Table 3. A Comparison of the Therapeutic Effects of Aspirin Between PL-12-Sensitive and PL-12 Insensitive Groups.[a]

			NIHSS		
Groups	Case	MAR (%)	On admission	On discharge	Improvement
PL-12 sensitive group	197	49.25 ± 7.23	4.87 ± 2.72	1.72 ±1.67	2.79 ± 1.43
PL-12 insensitive group	173	67.16 ± 5.32	5.25 ± 3.17	3.74 ± 2.78	1.33 ± 1.21
T		26.822	1.241	8.587	10.522
P		<0.001	0.215	<0.001	<0.001

Abbreviations: MAR, maximum platelet aggregation rate; NIHSS, National Institute of Health Stroke Scale.
a Values are presented as mean ± standard deviation.

Table 4. Comparison of PL-12 Platelet Function Detection, Platelet Counting and TEG Between PL-12-Sensitive and PL-12 Insensitive Groups.[a]

Groups	Case	Platelet counts($\times 10^9$/L)	R (min)	K (min)	Angel (deg)	MA (mm)	CI
PL-12 sensitive group	197	199.3± 37.31	6.22 ± 1.25	4.45 ± 0.52	44.38 ± 7.47	64.25 ± 8.39	1.39 ± 0.55
PL-12 insensitive group	173	212.4± 47.25	5.47 ± 1.31	2.28 ± 0.33	67.13 ± 8.11	72.14 ± 6.14	2.29 ± 0.79
t		2.976	5.631	47.173	28.080	10.200	12.836
P		0.003	<0.001	<0.001	<0.001	<0.001	<0.001

Abbreviations: R value, coagulation reaction time; K value, coagulation time; Angle, the rate of formation of a blood clot and the function of fibrinogen; MA, maximum thrombus amplitude; CI, coagulation index.
a Values are presented as mean ± standard deviation.

function was consistent with established methods. As illustrated in Table 4, the platelet count drop for the 197 patients in the PL-12-sensitive group was significantly lower than that of the 173 patients in the PL-12-insensitive group ($P < 0.05$). Furthermore, compared with the PL-12- insensitive group, the R value (coagulation reaction time) and the K value (coagulation time) were significantly higher in the PL-12-sensitive group. Angle (reflecting the rate of formation for blood clots and the function of fibrinogen), MA (the maximum thrombus amplitude), and CI (coagulation index), in the PL-12-sensitive group was significantly lower ($P < 0.05$).

Since MA parameters obtained from the TEG test represent the maximum strength of the clot and platelet counts are indicative of platelet activity, we further analyzed the correlation between MAR, as measured by the PL-12 analyzer, and the MA value detected by either TEG or platelet counts as determined by the platelet count drop method. Pearson correlation analysis showed that the MAR was positively correlated with MA values, as well as platelet counts ($r = 0.411$ and $r = 0.396$, respectively, $P < 0.001$ and $P < 0.05$, respectively, Figure 1A and B). These results indicated that the PL-12 analyzer was consistent with the platelet count drop method, and the TEG, in terms of measuring platelet function.

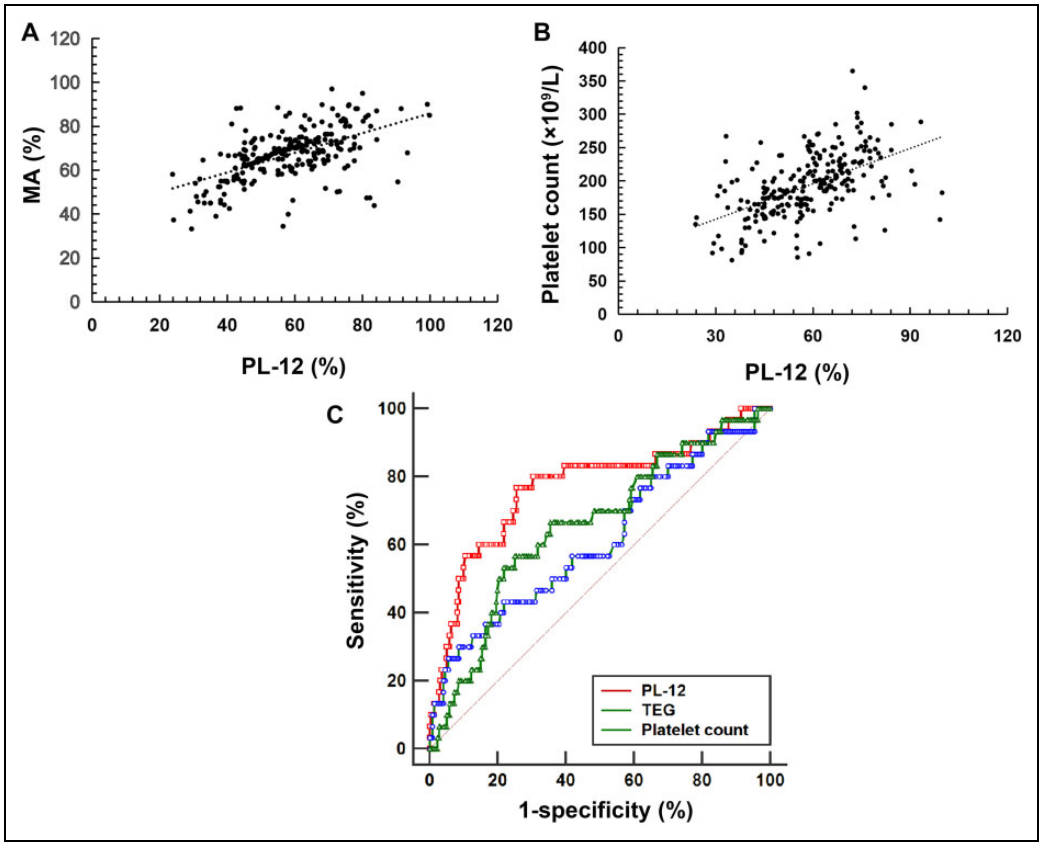

Figure 1. A, Correlation analysis between the PL-12 platelet function analyzer and MA parameters as derived from TEG. B, Correlation analysis between the PL-12 platelet function analyzer and the platelet count drop method. C, The predictive value of the PL-12 platelet function analyzer, thromboelastogram and platelet count drop method for determining end-point events by ROC curve analysis.

The Relationship Between the PL-12 Platelet Function Test and the Detection of Drug Resistance Gene

In order to explore the capability of the PL-12 analyzer to detect aspirin resistance in patients with ACI, we used PCR to detect polymorphisms in 5 SNPs in *GPIIb* (rs5911), *HO-1* (rs2071746), *PTGS1* (rs10306114), *COX-2* (rs20417) and *PEAR1* (rs12041331); these polymorphisms have all been reported to be involved with resistance to aspirin. In our study, 58 patients underwent genetic testing; the results are shown in Table 5. We found that the genotypic frequencies of *GPIIb* (rs5911), *HO-1* (rs2071746), *PTGS1* (rs10306114), *COX-2* (rs20417) and *PEAR1* (rs12041331) in the PL-12-sensitive and PL-12-insensitive groups were in accordance with the HWE equilibrium ($P > 0.05$). In addition, the frequency of AA + AG genotype as well as A-allele in *PEAR1* (rs12041331) was significantly higher in the PL-12-sensitive group when compared with the PL-12-insensitive group ($P < 0.05$). There were no significant differences in the genotypic frequencies of genotype or allele were found in *GPIIb* (rs5911), *PEAR1* (rs12041331), *PTGS1* (rs10306114) and *COX-2* (rs20417) when compared between the PL-12-sensitive group and the PL-12-insensitive group ($P > 0.05$). This result indicated that the A-allele in *PEAR1* (rs12041331) in the PL-12-sensitive group had increased notably and that this was associated with reduced platelet aggregation. Collectively, these data indicated that the automated PL-12 platelet function test is consistent with the results derived from drug resistance gene detection.

The Relationship Between Platelet Function, as Determined by the PL-12 Analyzer, and Patient Prognosis

After 12-months of follow-up, we investigated the occurrence of adverse events in ACI patients. As exhibited in Table 6, the mRS score in the PL-12-sensitive group was significantly lower than that in the PL-12-insensitive group at 3-months follow-up ($P < 0.05$). In addition, during the follow-up period, there were 9 cases of cerebral infarction recurrence in the PL-12-sensitive group (9/197, 4.57%) and 24 cases in the PL-12-insensitive group (24/173, 13.87%); consequently, there was a significant difference between the 2 groups with this respect ($P < 0.05$). With regard to bleeding events, there were significantly fewer cases in the PL-12-sensitive group (11/197, 5.58%) than in the PL-12-insensitive group (29/173, 16.76%) ($P < 0.05$). Mortality rates across the 2 groups presented a similar trend as with bleeding events; the number of deaths in the PL-12-sensitive group was significantly less (4/197, 2.03%) than that in the PL-12-insensitive group (14/173, 8.09%) ($P < 0.05$). Our results therefore suggest a correlation

Table 5. The Detection of Gene Polymorphisms that are Known to be Related to drug Resistance in the PL-12-Sensitive and PL-12-Insensitive Groups.[a]

Genes	PL-12 sensitive group (n = 41)	PL-12 insensitive group (n = 17)	$\chi 2$	*P*
GPIIb (rs5911)			0.884	0.347
AA	21 (0.51)	11 (0.65)		
CC+AC	20 (0.49)	6 (0.35)		
HO-1 (rs2071746)			1.568	0.211
AA	11 (0.27)	2 (0.12)		
TT+TA	30 (0.73)	15 (0.88)		
PTGS1 (rs10306114)			—	—
AA	41 (1.00)	17 (1.00)		
GG+AG	0 (0.00)	0 (0.00)	41	
COX-2 (rs20417)			—	—
GG	0 (0.00)	0 (0.00)		
CC+GC	41 (1.00)	17 (1.00)		
PEAR1 (rs12041331)			4.459	0.035
GG	12 (0.29)	10 (0.59)		
AA+AG	29 (0.71)	7 (0.41)		

Abbreviations: *GP IIb*, platelet glycoprotein (GP) IIb receptors; *HO-1*, heme oxygenase 1; *PTGS1*, prostaglandin-endoperoxide synthase 1; *COX-2*, cyclooxygenase 2; *PEAR1*, platelet endothelial aggregation receptor 1.
a Values are presented as n (%).

Table 6. Comparison of mRS Scores and Clinical Prognosis Between the 2 Groups During the Follow-Up Period.

Group	PL-12 sensitive group	PL-12 insensitive group	$t/\chi 2$	*P*
Case	197	173		
mRS score	0.34 ± 0.61	0.81 ± 0.96	5.688	<0.001
Cerebral infarction recurrence	9 (4.57%)	24 (13.87%)	9.816	<0.001
Bleeding events	11 (5.58%)	29 (16.76%)	11.939	<0.001
Death	4 (2.03%)	14 (8.09%)	7.314	<0.001

Abbreviation: mRS, modified Rankin Scale.
[a]Values are presented as mean ± standard deviation or n (%).

Table 7. The Predictive Value of These Methods for Determining End-Point Events at Follow-Up in Patients With ACI.

	AUC	95% CI	*P*	Cut-off value	Sensitivity	S
PL-12	0.748	0.704-0.792	<0.001	60.03%	0.874	0.574
TEG	0.622	0.573-0.671	<0.001	72.30%	0.504	0.772
Platelet count	0.611	0.561-0.661	<0.001	229	0.509	0.778
Gene polymorphisms					0.742	0.617

between platelet function, as measured by the PL-12 analyzer, and clinical prognosis in patients with ACI.

The Predictive Value of Different Methods for the Measurement of Platelet Function for End-Point Events

ROC curve analysis was used to determine the predictive value of the PL-12 analyzer, the TEG test, and the platelet count drop method with regard to the occurrence of adverse events and prognosis of ACI patients. Results demonstrated that the cut-off value for PL-12 platelet function for prognosis was 60.03% with a sensitivity of 87.4% and a specificity of 57.4%; the area under the curve (AUC) reached 0.748. With regard to the TEG test, the cut-off value for MA was 72.3 mm, for which the AUC was 0.622, the sensitivity was 50.4%, and the specificity was 77.2%. With regard to the platelet count drop method, the best cut-off value was 228×10^9 / L; the AUC was 0.611, and the sensitivity and specificity were 50.9% and 77.8%, respectively (Figure 1C and Table 7). As the AUC of the PL-12 system was the highest, and because the sensitivity and specificity were also the highest, it was evident that the PL-12 analyzer was superior to the TEG test and the platelet count drop method with regard to predicting the occurrence of adverse events and the prognosis of ACI patients.

Discussion

The measurement of platelet aggregation was started at the 1960s, utilizing plasma nephelometry.[32] Recently, the evaluation of platelet aggregation applied in monitoring of antiplatelet drug use and in *vitro* diagnostic detecting of platelet function has getting more attention.[33] With the progress of the technology, new methods emerge as the times require, for instance, the whole blood continuous counting platelet function analyzer PL-12, while few researches have been reported the application of PL-12 approach in ACI.

As an upgraded version of the PL-11 platelet function analyzer, the PL-12 platelet function analyzer is a new device detecting changes in impedance due to platelet aggregation on electrodes in whole blood samples,[19,34] which is more convenient than the detection of platelet aggregation with serum. Furthermore, results of each sample can obtained within 6 minutes, and dual detection in each sample hole can enable automatic quality control. Compared to the PL-11, the PL-12 can saves testing time and effort, improve detection efficiency, and certainly decreased the cost per measurement. Previous study has found that high platelet response to clopidogrel, determined by the PL-11, was related to incremental stent thrombosis after DES implantation.[34] In this study, PL-12 analyzer was used to detect the aspirin resistance in ACI patients, which showed that 173 patients (46.76%) of the patients tested had a MAR $\geq$ 60%, thus indicating that 46.76% patients had developed resistance to aspirin; this is consistent with a previous publication that reported a range of 5%–60% of patients to exhibit resistance to aspirin.[35] Additionally, Guan et al.[19] reported that high platelet reactivity (HPR) defined as MAR > 55% was highly agreement with HPR defined applying light transmission aggregometry (LTA, $r = 0.614$, $P < 0.01$), VerifyNow ($r = 0.829$, $P < 0.01$) and thromboelastography (TEG, $r = 0.697$, $P < 0.01$), which is also similar to our result, indicating PL-12 analyzer using in platelet aggregation evaluation of ACI patients was credibly.

As an traditional platelet method, the accuracy of the platelet count drop method in platelet aggregation assessment was still doubted. A study suggested that the platelet count drop method showed a moderate correlation with LTA,[14] while another study demonstrated that the platelet count drop method showed poor levels of correlation with LTA, the current gold standard.[36] Our study indicated that PL-12 enable to yield an exact result for the MAR instead of an ambiguous result by conducing the measurement several times intensively after mix the agonists with the citrated blood sample.

The TEG analyzer is a form of monitor which can reflect the whole process of coagulation, including platelet aggregation, fibrinolysis, and coagulation. The TEG has been used widely across the world to evaluate platelet activity and antiplatelet effects.[37,38] Wu et al.[39] utilize PL-12 to detect the platelet aggregation to explore the clinical significance of platelet MAR in patients with sepsis and found that the MAR of platelets is a good predictor of diagnosis of sepsis and the outcome of patients with severe sepsis. In this study, we found that MA value measured by TEG showed a good correlation with the MAR detected by the PL-12 system in ACI patients ($r = 0.322$, $P < 0.001$). Meanwhile, the PL-12 analyzer showed the largest AUC (0.748) with a sensitivity of 87.4% and a specificity of 57.4%; the best cut-off value was 60% and was superior to the TEG test and the platelet count method. Low sensitivity and specificity of the PL-12 analyzer probably due to the poor reproducibility of the platelet aggregation test and data changes within a certain range. Similar to our study, Guan et al.[19] emphasized that PL-11 had a higher diagnostic value than the LTA and VerifyNow in terms of monitoring the short-term efficacy of aspirin in healthy individuals when analyzed by ROC curves. Based on the above results, we believe that the PL-12 analyzer provides an better alternative approach to platelet count drop method and TEG method as PL-12 can simulate the environment of platelet aggregation better *in vivo* by continuously counting platelets before and after the addition of agonists.

Genomic polymorphism can increase the risk of bleeding after antiplatelet medical treatment.[17,18] Drug resistance gene detection in present study showed that the AA+AG genotype in the genetic polymorphism of *PEAR1* (rs12041331) was obviously more prevalent in the PL-12-sensitive group when compared to the PL-12-insensitive group ($P < 0.05$), means that an increased frequency of A allele in *PEAR1* (rs12041331) can dramatically reduce platelet aggregation in patients with ACI. Consistent with our study, Würtz et al.[40] previously illustrated that the A-allele of the rs12041331 SNP in the *PEAR1* gene was associated with reduced levels of platelet aggregation in aspirin-treated patients with coronary artery disease. In another study, Xiang et al.[41] reported that the G allele in *PEAR1* (rs12041331) was associated with enhanced platelet aggregation in response to multiple agonists, irrespective of whether drugs were present or not. Based on these results, we believe that the predictive value of the PL-12 analyzer for the prognosis of ACI patients was superior to the other methods tested herein. However, this might be related to the small sample size of this study, future studies should now aim to recruit a larger number of patients.

Collectively, our study offer a new superior technology for monitoring antiplatelet drug efficacy and for clinical prognosis of ACI patients, which has the advantages of simplicity, speed, and automation in platelet aggregation measurement, and has high value in clinical promotion. However, the true value of the PL-12 analyzer for the evaluation of clinical prognosis, and whether this method can be used on a large-scale in the clinic, still requires further validation.

Authors' Note

Cen Yue and Hanshui Chen conceived and supervised the study; Cen Yue designed experiments; Zhiwei Lin performed the experiments; Congxia Lu analyzed the data; Hanshui Chen wrote the manuscript. All authors reviewed the results and approved the final version of the manuscript. Zhiwei Lin is now affiliated to Department of Neurology, The First Hospital of Putian City, Putian, Fujian, China.

Declaration of Conflicting Interests

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