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Original Article

Correlation of platelet function with recurrent ischemic vascular events in stroke patients

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Abstract: Platelet activation and aggregation, as well as thrombosis, play an important role in the pathological and physiological processes of recurrent ischemic vascular events in stroke patients. The purpose of this study is to investigate the correlation of platelet function and recurrent ischemic vascular events in the secondary prevention of stroke. A total of 350 stroke patients within 72 h of onset from September 2014 to April 2015 were included in this study. The patients were administered aspirin starting from the day of admission. After 7-10 days, the platelet aggregation ratio was detected using method with continuous platelet counting, and the baseline data were recorded. The patients underwent a 6-month follow-up period, and the recurrent ischemic vascular events were observed. Logistic regression analysis was performed to obtain the risk factors for recurrent ischemic vascular events and the receiver operating characteristic (ROC) curve. The predictive value of the platelet aggregation ratio by method with continuous platelet counting detection for ischemic vascular events was analyzed. Among the 350 stroke patients, 52 patients had recurrent ischemic vascular events during the follow-up period. The proportion of the patients with recurrent ischemic events who also had diabetes (48.01% vs. 23.15%, $P<0.001$), low-density lipoprotein (LDL) cholesterol (3.02±0.74 vs. 2.74±0.72, $P=0.016$), an arachidonic acid-induced maximum platelet aggregation ratio (MAR-AA) (27.31±9.49 vs. 18.85±6.60, $P<0.001$), and an epinephrine-induced maximum platelet aggregation ratio (MAR-EPI) (61.26±13.02 vs. 51.41±14.81, $P<0.001$) was significantly higher than that of the patients in the no ischemic vascular event group. These increased parameters were determined to be the independent risk factors for the occurrence of ischemic vascular events in stroke patients. MAR-AA was most closely related to the occurrence of ischemic vascular events (*OR*=1.133, 95% *CI*: 1.080~1.188, $P<0.001$) and showed a good predictive value for ischemic events with an area under the ROC curve (AUC) of 0.803. The increase in MAR-AA was also an independent risk factor for stroke recurrence (*OR*=1.090, 95% *CI*: 1.037~1.145, $P=0.001$). In the secondary prevention of stroke, platelet function measured with the continuous platelet counting method is closely related to recurrent ischemic vascular events; thus, this parameter has a good predictive value for recurrent ischemic vascular events.

Keywords: Stroke, ischemic event, aspirin, platelet function, risk factor

Introduction

Platelet activation and aggregation, as well as thrombosis, play an important role in the pathological and physiological processes of recurrent ischemic vascular events in stroke patients. Aspirin is an effective antiplatelet aggregation drug. It can effectively prevent the occurrence of cardiovascular and cerebrovascular events, and it has been widely used for the secondary prevention of stroke [1]. However, recurrent ischemic strokes and other vascular events still occurred in 30-40% of stroke patients, even with long-term regular aspirin use [2, 3]. Therefore, the early identification of stroke patients with a high risk of recurrent ischemic events and the timely intervention to improve the prognosis and quality of life in these patients is vital. Currently, the secondary prevention of stroke utilizes hypertension, diabetes, high cholesterol, obesity, smoking, and alcoholism as indicators of the risk for recurrent ischemic vascular events. These indicators are relatively indirect and have limited predictive value. Studies have shown that platelet aggregation is closely related to the risk of recurrent ischemic vascular events in stroke patients [4]. Therefore, monitoring platelet aggregation will

be conducive to the prediction and prevention of recurrent ischemic vascular events; however, the available research in this field is lacking. Light transmittance aggregation (LTA) is currently the most widely used method of platelet function testing in clinical applications. LTA is considered the "gold standard" of platelet function detection [5, 6]; however, this method requires complicated procedures and shows poor reproducibility [7]. The PL-11 platelet analyzer is a new platelet function testing method with continuous platelet counting based on the Coulter principle. The operation of this analyzer is simple and fast. Studies have confirmed that the evaluation of platelet function using detection methods based on the principle of continuous platelet counting, including PL-11, show good reliability and accuracy, and thus a bright future in clinical applications [8-10]. The correlation of continuous platelet counting with the recurrence of ischemic vascular events in stroke patients and the predictive value of this method has not been fully elucidated. In this study, platelet function was evaluated using the platelet aggregation ratio calculated by PL-11 detection. The correlation of platelet function with the recurrence of ischemic events in stroke patients during the follow-up period was investigation. In addition, the predictive value of this method was determined. These data provide a new basis for the prediction of recurrent ischemic events in stroke patients.

Materials and methods

Study subjects

Stroke patients admitted to the Department of Neurology of Weihai Municipal Hospital, an affiliate of Binzhou Medical College within 72 h of onset from September 2014 to April 2015 were continuously selected. Inclusion criteria included (1) a stroke diagnosis confirmed by CT and MRI, (2) a case identified as cerebral infarction with large artery atherosclerosis (LAA) and small artery occlusion (SAO) according to the classification of Trial of ORG 10172 in Acute Stroke Treatment (TOAST) [11], and (3) an informed consent signed by the patients and their families. Exclusion criteria included patients with (1) Alzheimer's disease; (2) Fever, hypoxia, unconsciousness, or hemodynamic disorder at admission; (3) An allergy to acetylsalicylic acid; (4) Consumption of any drugs within one week of the stroke, in addition to aspirin, that would affect platelet aggregation function, such as non-steroidal antiplatelet drugs, low molecular weight heparin, and warfarin; (5) A platelet count $>450\times10^9$/L or $<100\times10^9$/L; (6) Severe liver or renal insufficiency, tumors, or disease of the immune or respiratory systems; and (7) Severe trauma or surgery within one month of the stroke. This study was approved by the ethics committee of our hospital, and all included patients signed the informed consent.

Research methods

Data collection: The general information (including age, gender, height, and weight), medical history (including history of hypertension, diabetes, coronary heart disease, and stroke), personal lifestyle history (such as smoking and drinking), medication history, family history, and the laboratory tests and imaging data (CTorMRI) of the patients were collected.

Treatment: The patients were administered 100 mg/d of aspirin (Aspirin®, BayerCorporation, Germany) beginning at the day of admission. Patients were also given medication to control blood pressure and blood glucose and lower lipids.

Detection of platelet aggregation ratio: The PL-11 platelet function analyzer was used with arachidonic acid (AA, at a concentration of 2 mg/ml), collagen (COL, at a concentration of 0.2 mg/ml), and epinephrine (EPI, at a concentration of 2 mg/ml). The standard sample for quality control was used with the C3 hematology analyzer (specific for the PL series platelet analyzer). All of the reagents were provided by Nanjing SINNOVA Medical Technology Co., Ltd., China. The platelet aggregation ratio of the patient was detected after the administration of aspirin for 7-10 days.

On the morning after fasting, 6 mL of venous blood was collected and transferred into a specialized test tube containing 660 μL of 3.8% sodium citrate. After mixing by inverting 3 times, the sample was immediately sent to the laboratory for testing. An aliquot of 0.5 mL of the citrate anticoagulated whole blood was transferred into the PL-11 test tube using a pipette, and the automatic detection was started. After the baseline plateletcount of the

whole blood sample was completed twice (the baseline value was calculated as the average of the two measurements), 40 μL of AA (the final concentration in the whole blood sample was 0.148 mg/mL), 40 μL of COL (the final concentration in the whole blood was 0.0148 mg/mL) or 40 μL of EPI (the final concentration in the whole blood was 0.148 mg/mL) was automatically added by the instrument, and the plateletcount for the blood sample was successively conducted with a certain time interval. When the minimum plateletcount was obtained, the result was automatically converted. The maximum aggregate ratio was automatically converted by the instrument based on the following equation: maximum platelet aggregation ratio (%) = (the baseline average platelet count-the minimum platelet count)/the baseline average platelet count ×100%. The analysis of all blood samples was completed within 2 h of collection, and all operations for the test were performed at room temperature (25°C).

Standards for quality control: The standard sample for the quality control of the C3 hematology analyzer was placed in an environment above 22°C prior to use and mixed using an automatic mixer for at least 30 minutes. After mixing, the liquid in the quality control tube was observed uniformity with no visible clumps or uneven precipitation on the wall. If the above requirement was not met, the standard sample was continuously mixed. After mixing, 500 μL of this sample was transferred into a detection beaker specific for the platelet analyzer, and the detection by the machine was executed with the correction of various parameters after the sample was mixed again. The sample was only applied in clinical testing after the requirements of all indexes were fully met. If the plateletcount error for the two samples before the addition of the aggregation-inducing agent exceeded 10%, then a retest was conducted.

Follow-up

Follow-up procedures: The patients were asked to continue taking aspirin (100 mg/d, with a meal in the evening) and to control their blood pressure, blood sugar and blood lipids after discharge from the hospital. Using the outpatient registration system for stroke screening in our department, the patients were followed up for six months by telephone calls, outpatient services, and home visits. All endpoint events were recorded.

Follow-up endpoint events: Multiple endpoint events were assessed: (1) the recurrence of stroke; (2) transient ischemic attack (TIA); (3) myocardial infarction; (4) peripheral vascular embolism or thrombus; (5) cerebral, gastrointestinal tract, eye, or skin hemorrhage; and (6) vascular death. Stroke recurrence was defined as an acute brain or retinal focal infarction accompanied with one of the following circumstances: (1) acute onset of a new focal neurological deficit with clinical signs or radiographic evidence for ≥24 hours induration, with the exclusion of other non-ischemic causes (e.g., brain infection, brain trauma, brain tumors, epilepsy, severe metabolic disorders, or degenerative neurological diseases); (2) acute cerebralor retinal ischemic events with focal symptoms or signs with aduration of <24 hours and radiographic evidence of a new infarction, with the exclusion of other non-ischemic causes; or (3) progression of the original vasogenic ischemic stroke with a duration ≥24 hours and new ischemic changes on a head MRI or CT that was significantly different from the original event. Myocardial infarction was defined as the increase in serum cardiac markers (mainly troponin) (at least higher than 99% of the upper limit of the reference value) with at least one of the following clinical indicators: (1) myocardial ischemic symptoms; (2) new onset of ischemic electrocardiographic changes (new ST-T changes or blockage at the left bundle branch); (3) formation of pathological Q waves on the electrocardiogram; (4) new loss of myocardial activity or new onset of regional wall motion abnormalities revealed by radiographic evidence; and (5) intracoronary thrombus confirmed by coronary angiography or autopsy. Vascular death was defined as death due to ischemic stroke, myocardial infarction, congestive heart failure, pulmonary embolism, sudden death, or arrhythmia.

Statistical analysis

SPSS13.0 statistical software (SPSS, Chicago, IL) was used for the statistical analysis. The normally distributed measurement data were represented as the mean ± standard deviation ($\bar{x} \pm s$), and the results of the two groups were compared using a ttest. The counting data were represented as a ratio, and the results of the two groups were compared using a χ^2 test. Multivariate logistic regression analysis was used to investigate the risk factors for ischemic

Table 1. Comparison of the general information and risk factors of the ischemic event group and the no ischemic event group during the follow-upperiod

Variable	Ischemic event group n=52	No ischemic event group n=298	*T* value or χ^2 value	*P* value
Clinical parameters				
Age (years)	63.75±8.44	63.68±10.01	0.044	0.965
Female (cases, %)	31 (59.62)	155 (52.01)	1.028	0.311
BMI	24.8±2.93	24.3±3.26	0.969	0.103
Smoking (cases, %)	13 (25.00)	63 (21.14)	0.388	0.533
Drinking (cases, %)	11 (21.15)	50 (16.78)	0.589	0.443
NIHSS score at admission	8.5±4.8	7.2±3.9	0.701	0.432
Laboratory data				
Platelet count ($\times 10^9$/L, $\bar{x}\pm s$)	245±72.53	237±68.76	0.437	0.631
Mean platelet volume (fL, $\bar{x}\pm s$)	10.89±1.01	10.60±1.21	0.401	0.697
Total cholesterol (mmol/L, $\bar{x}\pm s$)	4.46±1.13	4.30±1.14	0.955	0.340
Triglycerides (mmol/L, $\bar{x}\pm s$)	1.46±0.72	1.52±1.02	-0.376	0.707
LDL cholesterol (mmol/L, $\bar{x}\pm s$)	3.02±0.74	2.74±0.72	2.410	0.016
D-dimer (mg/L, $\bar{x}\pm s$)	1.23±1.32	1.01±1.41	0.672	0.532
Maximum platelet aggregation ratio (7-10 days after admission) (%)				
MAR-EPI	61.26±13.02	51.41±14.81	4.498	0.000
MAR-COL	37.97±16.17	31.80±13.82	2.896	0.004
MAR-AA	27.31±9.49	18.85±6.60	6.173	0.000
Medical history (cases, %)				
Hypertension	35 (67.31)	195 (65.44)	0.069	0.793
Diabetes	25 (48.01)	69 (23.15)	14.000	0.000
Coronary Heart Disease	16 (30.77)	86 (28.86)	0.078	0.780
History of stroke or TIA	10 (19.23)	54 (18.12)	0.037	0.848
Type of stroke (cases, %)				
LAA	35 (67.31)	189 (63.42)	0.290	0.590
SAO	17 (32.69)	109 (36.58)		

BMI, body mass index; NIHSS, National Institute of Health stroke scale; LDL, low-density lipoprotein; MAR-EPI, epinephrine-induced maximum platelet aggregation ratio; MAR-COL, collagen-induced maximum platelet aggregation ratio; MAR-AA, arachidonic acid-induced maximum platelet aggregation ratio; LAA, large artery atherosclerosis; SAO, small artery occlusion.

vascular events and stroke recurrence. The AUC was used to assess the predictive value of the maximum platelet aggregation ratio for ischemic vascular events and stroke recurrence and determine the boundary values for the prediction of ischemic vascular events and stroke recurrence. Differences with a $P<0.05$ was considered statistically significant.

Results

Endpoint events

During the study period, 373 patients were identified as candidates. A total of 15 patients did not continue the aspirin antiplatelet therapy during the follow-up, and 8 patients changed to other antiplatelet drugs during the follow-up. The remaining 350 patients were included in the final analysis. Major ischemic vascular events occurred in 52 patients, including 38 cases of recurrent stroke, 5 cases of new-onset transient ischemic attack, 5 cases of myocardial infarction, 3 cases of peripheral vascular thrombosis or occlusion, 1 case of vascular death, 2 cases of cerebral hemorrhage, and 1 case of gastrointestinal hemorrhage with no major hemorrhage event. The included patients were divided into two groups based on whether ischemic vascular events (including stroke recurrence, new TIA, myocardial infarc-

Table 2. Multivariate logistic regression analysis for ischemic vascular events

Independent variable	Regression coefficient *B*	Standard error *S.E.*	*P* value	*OR* value	95% *CI*
Diabetes	0.863	0.365	0.018	2.370	1.158~4.850
LDL cholesterol	0.426	0.228	0.062	1.531	0.979~2.394
MAR-AA	0.125	0.024	0.000	1.133	1.080~1.188
MAR-EPI	0.038	0.012	0.002	1.039	1.014~1.042
MAR-COL	0.017	0.012	0.186	1.017	0.992~1.046

LDL, low-density lipoprotein; MAR-AA, arachidonic acid-induced maximum platelet aggregation ratio; MAR-EPI, epinephrine-induced maximum platelet aggregation ratio; MAR-COL, collagen-induced maximum platelet aggregation ratio.

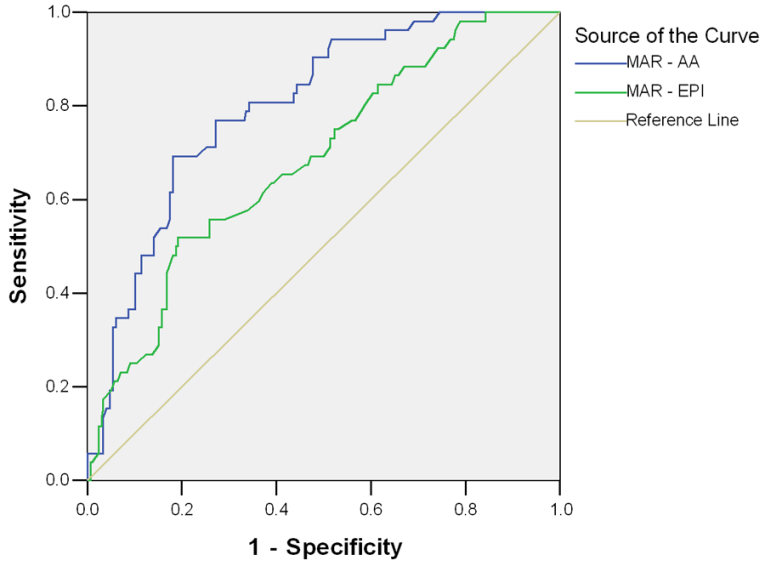

Figure 1. Comparison of the ROC curves for MAR-AA and MAR-EPI detected by continuous platelet counting method in the prediction of ischemic vascular events.

tion, peripheral vascular thrombosis or occlusion, and vascular death) occurred during the period of aspirin administration. A total of 52 patients were assigned to the ischemic vascular event group (ischemic event group), and 298 patients were assigned to the no ischemic vascular event group (noischemic event group).

Univariate analysis for the occurrence of ischemic vascular events

The general information and risk factors for the 2 groups are compared in **Table 1**. A univariate analysis showed that age, gender, body mass index, smoking, and total cholesterol level were not significantly different between the 2 groups ($P>0.05$). The differences in the level of low-density lipoprotein (LDL) cholesterol, diabetes, arachidonic acid-induced maximum platelet aggregation ratio (MAR-AA), collagen-induced maximum platelet aggregation ratio (MAR-COL), and epinephrine-induced maximum platelet aggregation ratio (MAR-EPI) between the two groups were statistically significant ($P<0.05$).

Multivariate analysis for the occurrence of ischemic vascular events

A multivariate logistic regression analysis was carried out with the ischemic vascular events as the dependent variable. The variables of diabetes, LDL cholesterol, MAR-AA, MAR-COL, and MAR-EPI were used as the independent variables. The results showed that diabetes (*OR*= 2.370, 95% *CI*: 1.158~4.850, *P*=0.018), MAR-AA (*OR*=1.133 95% *CI*: 1.080~1.188, *P*< 0.001), and MAR-EPI (*OR*= 1.039, 95% *CI*: 1.014~1.042, *P*=0.002) were the risk factors for ischemic vascular events (**Table 2**).

The predictive value of the platelet aggregation ratio determined by the ROC curve for ischemic vascular events

The AUC was used to evaluate the predictive value of the MAR-AA and MAR-EPI detected by PL-11 for vascular events (**Figure 1**). The AUC for MAR-AA was 0.803 (95% *CI*: 0.744-0.861, *P*<0.001). The AUC for MAR-EPI was 0.683 (95% *CI*: 0.606-0.759, *P*<0.001). The best cutoff value for the diagnosis of ischemic vascular events based on the maximum Youden index calculated by the ROC curve was 21.05% for the MAR-AA and 60.85% for the MAR-EPI.

Multivariate analysis of stroke recurrence

The general information and risk factors of the patients in the stroke recurrence group and the nostroke recurrence group during the follow-up are compared in **Table 3**. A multivariate logistic regression analysis was carried out with the occurrence of stroke during the follow-

Table 3. Comparison of the general information and risk factors of the strokerecurrence group and the nostroke recurrence group during the follow-upperiod

Variable	Stroke recurrence group n=38	No stroke recurrence group n=312	*T* value or χ^2 value	*P* value
Age (years)	64.26±8.01	63.6±10.05	0.377	0.706
Female (cases, %)	24 (63.16)	162 (51.92)	1.717	0.190
Hypertension (cases, %)	26 (68.42)	204 (65.38)	0.139	0.710
Diabetes (cases, %)	21 (55.26)	73 (23.40)	17.510	0.000
Smoking (cases, %)	10 (26.32)	63 (20.19)	0.769	0.380
LDL cholesterol (mmol/L, $\bar{x}\pm s$)	3.17±0.68	2.74±0.74	3.363	0.001
Total cholesterol (mmol/L, $\bar{x}\pm s$)	4.57±0.99	4.29±1.16	1.459	0.146
MAR-AA (%)	26.99±8.28	19.27±7.20	6.135	0.000
MAR-EPI (%)	63.52±13.38	51.58±14.64	4.789	0.000
MAR-COL (%)	41.08±16.71	31.70±13.71	3.883	0.002

LDL, low-density lipoprotein; MAR-AA, arachidonic acid-induced maximum platelet aggregation ratio; MAR-EPI, epinephrine-induced maximum platelet aggregation ratio; MAR-COL, collagen-induced maximum platelet aggregation ratio.

Table 4. Multivariate logistic regression analysis for stroke recurrence

Independent variable	Regression coefficient *B*	Standard error *S.E.*	*P* value	*OR* value	95% *CI*
Diabetes	1.002	0.409	0.014	2.724	1.223~6.067
LDL cholesterol	0.756	0.262	0.004	2.130	1.274~3.561
MAR-AA	0.086	0.025	0.001	1.090	1.037~1.145
MAR-EPI	0.052	0.016	0.001	1.054	1.022~1.086
MAR-COL	0.034	0.014	0.018	1.034	1.006~1.064

LDL, low-density lipoprotein; MAR-AA, arachidonic acid-induced maximum platelet aggregation ratio; MAR-EPI, epinephrine-induced maximum platelet aggregation ratio; MAR-COL, collagen-induced maximum platelet aggregation ratio.

up period as the dependent variable. The variables of diabetes, LDL cholesterol, MAR-AA, MAR-COL, and MAR-EPI were used as the independent variables. The results showed that diabetes (*OR*=2.724, 95% *CI*: 1.223~6.067, *P*=0.014), LDLcholesterol (*OR*=2.130, 95% *CI*: 1.274~3.561, *P*=0.004), MAR-AA (*OR*=1.090, 95% *CI*: 1.037~1.145, *P*=0.001), MAR-COL (*OR*=1.034, 95% *CI*: 1.006~1.064, *P*=0.018), and MAR-EPI (*OR*=1.054, 95% *CI*: 1.022~1.086, *P*=0.001) were the risk factors for stroke recurrence (**Table 4**).

The predictive value of the platelet aggregation ratio determined by the ROC curve for stroke recurrence

The AUC was used to evaluate the predictive value of the MAR-AA and MAR-EPI detected by PL-11 for stroke recurrence (**Figure 2**). The AUC for MAR-AA was 0.810 (95% *CI*: 0.746-0.875, *P*<0.001). The AUC for MAR-EPI was 0.684 (95% *CI*: 0.602-0.766, *P*<0.001). The AUC for MAR-COL was 0.677 (95% *CI*: 0.589~0.765, *P*< 0.001). The best cutoff value for the diagnosis of stroke recurrence based on the maximum Youden index calculated by the ROC curve was 23.05% for the MAR-AA, 62.95% for the MAR-EPI, and 30.55% for the MAR-COL.

Discussion

In recent years, many studies showed that platelet activity after aspirin treatment was effectively suppressed. In turn, platelet reactivity, a feature closely related to stroke prognosis, is enhanced [12, 13]. In this study, we provide additional evidence that an arachidonic acid-induced maximum platelet aggregation ratio (MAR-AA), and an epinephrine-induced maximum platelet aggregation ratio (MAR-EPI) in stroke patients with recurrent ischemic events was significantly higher than that of the patients in the no ischemic vascular event group. These increased parameters were determined to be the independent risk factors for the occurrence of ischemic vascular events in stroke patients. Furthermore, MAR-AA showed a good predictive value for ischemic events with an area under the ROC curve of 0.803.

Stroke recurrence is related to multiple risk factors. High LDL cholesterol and diabetes are currently recognized in China and other countries as the risk factors for stroke and recurrent stroke [14, 15]. Consistent with previous studies [14, 15], our study found that the proportion of patients with diabetes and high LDL choles-

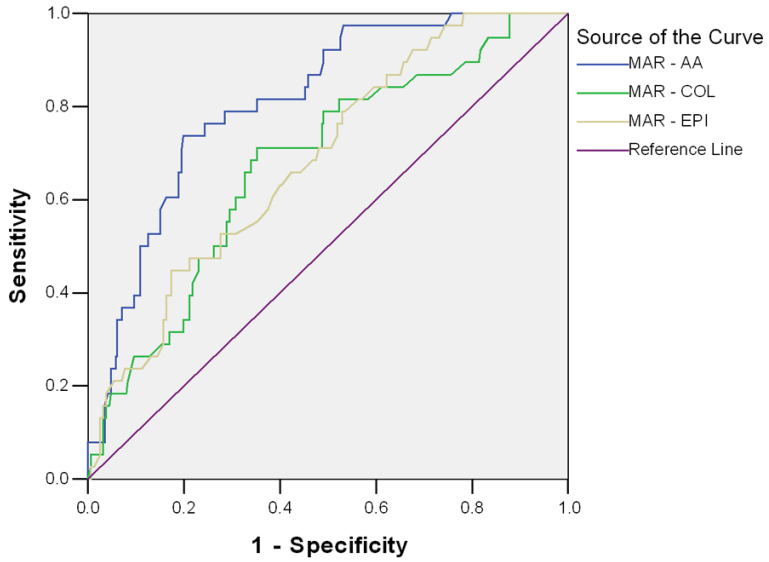

Figure 2. Comparison of the ROC curves for MAR-AA, MAR-COL, and MAR-EPI detected by continuous platelet counting method in the prediction of stroke recurrence.

terol levels was significantly increased in the stroke recurrence group when compared with the no stroke recurrence group. These increased parameters were independent risk factors for recurrent stroke. To date, studies on the risk factors for predicting the recurrence of stroke are mainly focused on the history of stroke, hypertension, diabetes, high homocysteine levels, high-sensitivity C-reactive protein (hsCRP) levels, symptom aticintracranialarterialstenosis, and the type of stroke [14, 16, 17]. However, use of these risk factors is relatively indirect for predicting the recurrence of stroke, and their predictive values are very limited. Current research suggests the aforementioned risk factors lead to vascular intimal injury. Specifically, the exposure of basal collagen and microfibers under the intima causes platelet activation and enhanced platelet reactivity, which leads to the occurrence of platelet adhesion, aggregation, release, and thrombosis. Therefore, the direct assessment of platelet reactivity by detecting platelet aggregation is useful for the prediction of stroke recurrence. Recent studies suggest that the detection of platelet aggregation and recurrent ischemic vascular events in stroke patients are closely related [18, 19]. In a study of 105 ischemic stroke patients by Schwammenthal et al. [18], all patients received aspirin therapy after admittance to the hospital, and platelet aggregation was assessed using LTA. The patients were divided into groups with a good aspirin response, a partial aspirin response, and no aspirin response. The 11.5-month follow-up showed that stroke severity and the incidence of clinical adverse events in the groups with a partial aspirin response and no aspirin response were significantly higher when compared with the good aspirin response group ($P<0.05$). A study of aspirin therapy in 643 Chinese stroke patients with an average follow-up of 19.4 months showed that the incidence of stroke recurrence, myocardial infarction, and all-cause mortality in the patients with a maximum platelet aggregation ratio ≥70% was significantly increased ($P<0.001$). This study also found that the risk of vascular events in patients with a maximum platelet aggregation ratio ≥70% was significantly increased (81.55% vs. 13.28%; *RR* 3.223, 95% CI: 1.155-7.256, $P<0.001$) [19]. Currently, the majority of worldwide correlation studies on platelet function detection and the poor prognosis of stroke patients were based on the pre-screening of aspirin resistance (AR) patients. After this screening, the impact of AR on the recurrence of ischemic vascular events and its predictive value were evaluated. However, the AR diagnostic criteria with different detection methods were not standardized, and the AR diagnostic cutoffs were mainly determined using cross-sectional and case-control studies [4]. Our study is the first prospective study to detect the platelet aggregation ratio by PL-11 and directly evaluate the correlation of platelet function with the recurrence of ischemic vascular events. We also showed the important clinical significance for the prediction and prevention of the recurrent ischemic events in stroke patients using platelet function. Our study found that the MAR-AA was significantly higher in the ischemic event group during the follow-up period, and this increase was a relatively independent risk factor for the occurrence of ischemic vascular events in stroke patients. The MAR-AA showed

a good predictive value for ischemic events with an AUC of 0.803. Our study also found that an increased MAR-AA was an independent risk factor for stroke recurrence. The MAR-AA showed a good predictive value for the recurrence of stroke with an AUC of 0.810.

EPI is a weak inducer of platelets. EPI effects depend on the secretion of platelets after aggregation and activation; thus, EPI induces the aggregation process without causing the deformation of platelets. EPI can bind to the EPIα2 receptor to activate phospholipase C and generate inositol triphosphate (IP3) and diacylglycerol (DG). Using the IP3 and DG pathways, protein kinases can be activated to inhibit adenylate cyclase, promote the release of calciumions in the endoplasmic reticulum, and activate phospholipase A2, which eventually induces platelet aggregation by the AA pathway [20]. The increased MAR-EP suggests that aspirin does not effectively inhibit the metabolism of AA. AA may still generate prostagland in H_2 and promote vasoconstriction and platelet aggregation through the cyclooxygenase-2 (COX-2) alternative pathway with a low inhibition rate of aspirin and the non-enzymatic lipid oxidation pathway. This alternative mechanism may explain the occurrence of adverse ischemic events. Our results showed that the MAR-EPI of the ischemic event group was significantly increased when compared with the no ischemic event group. This increase was closely related to the number of ischemic vascular events. In addition, the increased MAR-EPI was an independent risk factor for the recurrence of stroke. The prediction accuracy of ischemic stroke recurrence for MAR-EPI was evidenced by an AUC of 0.684. Therefore, the results of our study suggest that the detection of the platelet aggregation ratio using PL-11 in the secondary prevention of stroke can effectively predict the risk of ischemic vascular events.

Limitations

First, the follow-up period was relatively short. This study investigated the recurrent ischemic vascular events within six months of the original stroke. The correlation of platelet function with ischemic vascular events and its predictive value remains to be verified by studies with a long-term follow-up. Second, the sample size was small. Multi-center and large-scale clinical studies are needed to clarify the impact of platelet aggregation on recurrent ischemic vascular events in stroke patients. Third, the current detection of platelet aggregation function was based on the PL-11 in China and other countries. The prediction standards for recurrent ischemic vascular events in stroke patients are not yet developed, and larger, more in-depth studies are needed to establish these criteria and guide the treatment.

Conclusion

In the secondary prevention of stroke, platelet function measured with the continuous platelet counting method is closely related to recurrent ischemic vascular events; thus, this parameter has a good predictive value for recurrent ischemic vascular events.

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Disclosure of conflict of interest

None.

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