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Roles of light transmission aggregometry and CYP2C19 genotype in predicting ischaemic complications during interventional therapy for intracranial aneurysms

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ABSTRACT

Background and purpose Light transmission aggregometry (LTA) and CYP2C19 genotype analysis are commonly used to evaluate the antiplatelet effects of clopidogrel during the interventional treatment of intracranial aneurysms. The aim of this study was to determine which test can predict ischaemic events during these treatments.

Methods Patient demographic information, imaging data, laboratory data and ischaemic complications were recorded. LTA and CYP2C19 genotype results were compared, and multiple linear regression was performed to examine factors related to platelet reactivity. Multivariate regression analysis was performed to determine whether LTA and CYP2C19 could predict ischaemic complications and to identify other clinical risk factors. Receiver operating characteristic curve analysis was conducted to calculate the cut-off value for predicting ischaemic complications. A subgroup analysis was also performed for different CYP2C19 genotype metabolisers, as well as for patients with flow diverters and traditional stents.

Results A total of 379 patients were included, of which 22 developed ischaemic events. Maximum platelet aggregation induced by ADP (ADP-MPA) could predict ischaemic events (p<0.001; area under the curve, 0.752 (95% CI 0.663 to 0.842)), and its cut-off value was 41.5%. ADP-MPA (p=0.001) and hypertension duration >10 years (p=0.022) were independent risk factors for ischaemic events, while the CYP2C19 genotype was not associated with ischaemic events. In the subgroup analysis, ADP-MPA could predict ischaemic events in fast metabolisers (p=0.004) and intermediate metabolisers (p=0.003). The cut-off value for ischaemic events was lower in patients with flow diverters (ADP-MPA=36.4%) than in patients with traditional stents (ADP-MPA=42.9%).

Conclusions ADP-MPA can predict ischaemic complications during endovascular treatment of intracranial aneurysms. Patients with flow diverters require stronger antiplatelet medication than patients with traditional stents.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Light transmission aggregometry (LTA) and CYP2C19 genotype testing are used to evaluate the antiplatelet effect of clopidogrel. We compared the predictive ability of the two methods for ischaemic complications in interventional therapy for intracranial aneurysms.

WHAT THIS STUDY ADDS

⇒ CYP2C19 genotypes were unrelated to ischaemic events. Maximum platelet aggregation induced by ADP (ADP-MPA) could predict the risk of ischaemic events. The cut-off value of ADP-MPA for the occurrence of ischaemic events in patients with flow diverters was lower than in patients with traditional stents.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ For stent neurointerventional therapy of unruptured intracranial aneurysms, this study could provide a promising method for adjusting appropriate antiplatelet therapy.

INTRODUCTION

Endovascular therapy is currently one of the most commonly used methods of treating unruptured intracranial aneurysms (UIAs).[1] Intracranial stents, especially flow diverters, have been used to cure many wide-necked or intractable aneurysms.[2] However, studies have demonstrated that stents can increase the risk of ischaemic complications, which is the main reason for the poor prognosis of these patients.[3–5] Dual antiplatelet therapy (DAT; 100 mg of aspirin and 75 mg of clopidogrel daily) is a recognised method of preventing thrombotic complications.[6 7] However, some patients with resistance to antiplatelet therapy (mainly clopidogrel) show high on-treatment platelet reactivity (HTPR), which could be due to CYP2C19 genotype polymorphisms, drug interactions, and chronic diseases such as diabetes and chronic kidney disease.[8–11] Therefore, identifying patients with HTPR with corresponding adjustments

to antiplatelet drugs is key to reducing ischaemic events during neurointerventional therapy.

Light transmission aggregometry (LTA) is the gold standard for evaluating platelet aggregation function.[12] In a randomised controlled trial, replacement of clopidogrel with ticagrelor in patients with HTPR tested by LTA was shown to effectively reduce ischaemic events associated with neurointervention for UIAs.[13] Before the results were published, antiplatelet management in patients with HTPR was disorganised and depended on the preferences of the neurointerventionists involved: some opted for the enhancement or replacement of DAT, some opted for the postoperative addition of short-term low-molecular-weight heparin or tirofiban, and some did not consider intervention necessary. After the results were published, our centre began routine adjustment of antiplatelet drugs for patients with HTPR.

In a previous study, the diagnostic criteria for HTPR included the maximum platelet aggregation rate induced by ADP (ADP-MPA) ≥50% or the MPA rate induced by arachidonic acid (AA-MPA) ≥20% based on experience in coronary interventional therapy.[13 14] Even so, the definition of HTPR in neurointerventional therapy remains controversial, and there is a lack of individualised standards for patients with flow diverters. In addition to LTA, CYP2C19 genotype testing is also used to evaluate the antiplatelet effect of clopidogrel, but more evidence is needed to better understand the role of CYP2C19 genotyping in neurointervention.[8 15] Therefore, in this study, we selected patients with UIAs who underwent intracranial stent implantation procedures. These patients did not receive standard antiplatelet adjustment regimens because they underwent treatment before the drug adjustment consensus was established. We analysed the relationships between LTA and CYP2C19 genotype test results and compared the predictive ability of the two methods for ischaemic complications.

METHODS

Patient selection

This study was a single-centre retrospective study approved by the institutional review board. Based on the following inclusion/exclusion criteria, we collected patients with UIAs treated at the neurointerventional centre in our hospital from September 2020 to August 2021. All patients agreed to undergo the operation and signed an informed consent form.

The inclusion criteria were: (1) presence of UIA and treatment with intracranial stents; (2) performance of CYP2C19 genotype testing and LTA before the operation; (3) age of 18–80 years; (4) presence of modified Rankin Scale ≤2 before the operation and (5) administration of standard DAT before the procedure.

The exclusion criteria were: (1) simultaneous treatment for other cerebrovascular diseases, such as intracranial arteriovenous malformation and intracranial arteriovenous fistula; (2) allergy to aspirin or clopidogrel, or taking other antiplatelet drugs before the procedure; (3) high risk of bleeding, such as symptomatic intracranial haemorrhage or active gastric ulcer, or presence of bleeding tendency or coagulopathy; (4) thrombocytopenia (platelet count <100 $\times 10^9$/L before enrolment); (5) pregnancy or lactation among women; (6) presence of liver disease, kidney disease, congestive heart failure, malignant tumours or other malignant diseases; and (7) participation in other clinical studies.

Medical record review

We recorded the patients' basic characteristics, drug management, laboratory data, imaging data and procedure complications. The primary clinical outcome was ischaemic events within 1 month, including stent thrombosis, ischaemic stroke or transient ischaemic attack (TIA). Stent thrombosis was defined as thrombosis at the stent site confirmed by digital subtraction angiography after stent implantation. Ischaemic stroke was defined as an episode of neurological dysfunction caused by focal cerebral, spinal or retinal infarction.[16] TIA was defined as a transient episode of neurological dysfunction caused by focal brain, spinal or retinal ischaemia, without evidence of associated acute focal infarction of the brain.[17] If patients developed neurological deterioration, CT and MRI examinations were performed. Each ischaemic event was independently adjudicated by two neurointerventionists.

CYP2C19 genotype testing and LTA

CYP2C19 genotype testing was conducted on outpatients or upon admission. The CYP2C19 genetic polymorphisms were extracted from blood samples and detected using the PCR-restriction fragment length polymorphisms technique. CYP2C19 is highly polymorphic, with some alleles leading to loss of function and others leading to gain of function. Consistent with a previous classification method based on the CYP2C19*1, CYP2C19*2, CYP2C19*3 and CYP2C19*17 alleles,[18] the patients were divided into four metabolic types: those with *1/*1 haplotype were classified as fast metabolisers (FMs); those with *1/*17 and *17/*17 haplotypes were classified as ultra-fast metabolisers (UMs); those with *1/*2, *2/*17, *1/*3 and *3/*17 haplotypes were classified as intermediate metabolisers (IMs); and those with *2/*2, *2/*3 and *3/*3 haplotypes were classified as poor metabolisers (PMs).

Patients underwent LTA 1 day before the operation. LTA was conducted by a turbidimetric method using platelet-rich plasma in a four-channel aggregometer (AG800; Techlink Biomedical, Beijing, China). Briefly, 5 µmol/L ADP and 1 mg/mL AA were used to specifically and sensitively evaluate the effect of clopidogrel and acetylsalicylic acid on platelets, respectively. Curves were recorded for 6 min, and the MPA was defined as the per cent change in light transmittance.

Procedure details

Patients were administered DAT once a day for at least 5 days before the operation. All operations were performed under general anaesthesia. The activated clotting time was used to guide heparin administration during the process, with a target of 250–300 s, and typical doses of 3000–5000 U at the start of the procedure and 1000 U/hour. After measuring the anatomical parameters of the aneurysm and its parent artery, appropriate stent implantation was identified. Patients continued to take DAT daily. Patients with non-flow diverter stents took aspirin and clopidogrel for at least 6 months and 6 weeks, respectively, while patients with flow diverters took aspirin for life and clopidogrel for at least 3 months.

Statistical analysis

All statistical analyses were performed using SPSS V.25 (IBM Corp) and GraphPad Prism V.8.0 (GraphPad, La Jolla, California, USA). Values with $p<0.05$ were considered statistically significant. Data were presented as mean and range for continuous variables and as frequency for categorical variables. An unpaired t-test was used to compare platelet reactivity among different metabolisers. Multiple linear regression was performed to determine factors affecting platelet aggregation function, with dummy variables set for CYP2C19 metabolism type and FMs used as a reference. Binary logistic regression was performed to identify risk factors for ischaemic complications, with factors having $p<0.1$ subjected to a multivariate logistic regression analysis. Receiver operator characteristic (ROC) curve analysis was conducted to determine the ability of LTA to distinguish between patients with and without ischaemic events. The optimal cut-off value was obtained by calculating the maximum value of the Jorden index.

RESULTS

Basic patient information and aneurysm information

A total of 379 patients were included in the study cohort based on the inclusion and exclusion criteria. The clinical characteristics, laboratory data and procedural data of the patients are shown in table 1. The mean age was 56.7±10.5 years (range, 19–80 years), 244 (64.4%) patients were women, 194 (51.2%) patients had hypertension, the duration of hypertension was 10.5±8.5 years (range, 1–42 years), 68 (17.9%) patients were smokers, the mean maximum aneurysm size was 7.9±5.8 mm (range, 1.8–36.5 mm), 58 (15.3%) aneurysms were located in the posterior circulation and 199 (52.5%) patients were treated with flow diverters.

Table 1 Demographic, clinical and procedural characteristics of the patients

Variables	Value
Cases	379
Age, years; mean (SD)	56.7±10.5
Female (%)	244 (64.4)
Smoking (%)	68 (17.9)
Drinking (%)	34 (9.0)
Hypertension (%)	194 (51.2)
Hyperlipidaemia (%)	46 (12.1)
Diabetes (%)	42 (11.1)
Coronary artery disease (%)	22 (5.8)
Cerebrovascular stenosis (%)	65 (17.2)
Ischaemic cerebrovascular disease (%)	31 (8.2)
Red cell count, $\times10^{12}$/L; mean (SD)	4.6±5.2
White cell count, $\times10^{9}$/L; mean (SD)	8.1±26.7
Platelet count, $\times10^{9}$/L; mean (SD)	242.3±61.8
APTT, s; mean (SD)	30.4±3.3
INR, %; mean (SD)	1.0±0.1
Posterior circulation UIAs (%)	58 (15.3)
Maximum size, mm; mean (SD)	7.9±5.8
Multiple aneurysms (%)	78 (20.6)
Non-saccular aneurysms (%)	42 (11.1)
Flow diverters (%)	199 (52.5)
LTA	
ADP-MPA, %; mean (SD)	36.6±14.0
AA-MPA, %; mean (SD)	9.8±7.7
Metabolic types	
Ultra-fast metabolisers (%)	5 (1.3)
Fast metabolisers (%)	155 (40.9)
Intermediate metabolisers (%)	169 (44.6)
Poor metabolisers (%)	50 (13.2)

AA-MPA, maximum platelet aggregation rate induced by arachidonic acid; ADP-MPA, maximum platelet aggregation rate induced by ADP; APTT, activated partial thromboplastin time; INR, international normalised ratio; LTA, light transmission aggregometry; UIAs, unruptured intracranial aneurysms.

LTA and CYP2C19 genotyping test results

The mean preoperative ADP-MPA was 36.6%±14% (range, 2.5%–78.7%) and the mean preoperative AA-MPA was 9.8%±7.7% (range, 1.2%–82.6%). The CYP2C19 genotype test results showed that there were 5 UMs (1.3%), 155 FMs (40.9%), 169 IMs (44.6%) and 50 PMs (13.2%). After comparing the results of the two detection methods, the ADP-MPA value significantly increased in patients who were *2/*3 loss-of-function allele carriers (figure 1). The average ADP-MPA value in IMs was significantly greater than in FMs (39.36% vs 31.62%, $p<0.001$), while the average value in PMs was significantly greater than in IMs (44.67% vs 39.36%, $p=0.018$), suggesting that loss-of-function alleles lead to a decrease in the antiplatelet aggregation effect of clopidogrel. However, when using the definition for HTPR in the previous study (ADP-MPA ≥50%), the results of the two tests were not completely consistent: in the 50 PMs, only 38% (19 of 50) had ADP-MPA ≥50%; of the 62 patients with ADP-MPA ≥50% detected by LTA, 32.3% (20 of 62) were PMs, but 85.4% (53 of 62) carried CYP2C19 loss-of-function alleles. In the multivariate linear regression analysis conducted

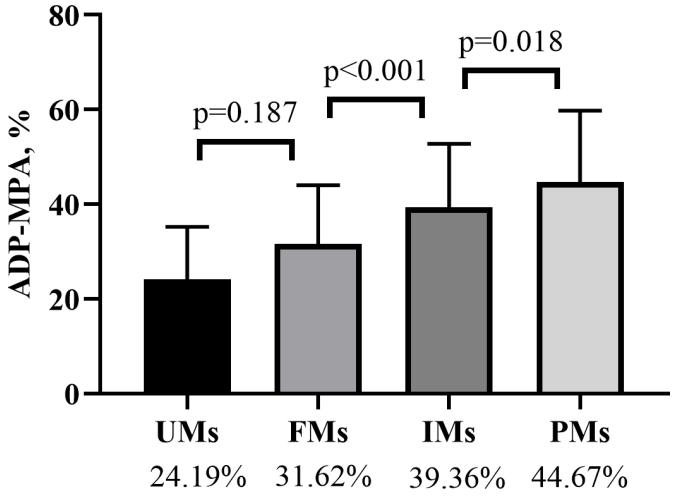

Figure 1 ADP-MPA values in different CYP2C19 gene metabolisers. ADP-MPA, maximum platelet aggregation rate induced by ADP; FMs, fast metabolisers; IMs, intermediate metabolisers; PMs, poor metabolisers; UMs, ultra-fast metabolisers.

to identify factors influencing platelet reactivity (table 2), the factors positively associated with ADP-MPA were age (p<0.001), platelet count (p=0.021), hypertension (p=0.023), IMs (p<0.001) and PMs (p<0.001).

Ischaemic complications

Twenty-two (5.8%) patients developed ischaemic events. Stent thrombosis occurred in two patients. Ischaemic stroke occurred in 18 patients (14 during hospitalisation and 4 after discharge). Two other patients developed transient neurological defects that were confirmed as TIAs.

Binary logistic regression analyses were conducted to identify factors associated with ischaemic events. In these analyses, seven parameters had values of p<0.1 (table 3) and were entered in the multivariate logistic regression analysis. Finally, ADP-MPA and hypertension duration >10 years were selected from all variables. ADP-MPA (p=0.001, OR=1.073) and hypertension duration >10 years (p=0.022, OR=3.009) were identified as independent predictors for ischaemic events (table 4). However, AA-MPA was not associated with ischaemic events (p=0.815), nor were CYP2C19 loss-of-function genotypes (IMs, p=0.212; PMs, p=0.249).

ROC curve analysis showed that the ADP-MPA cut-off value of 41.5% could distinguish patients with and without ischaemic events (figure 2; p<0.001; area under the curve (AUC) 0.752 (95% CI 0.663 to 0.842)), with a sensitivity of 81.8% (18 of 22 patients) and a specificity of 65.0% (232 of 357 patients). When patients were grouped according to the cut-off value, logistic regression analysis showed that patients with ADP-MPA ≥41.5% comprised 37.7% of the study population and had a >5-fold higher risk of ischaemic complications than patients with ADP-MPA <41.5% (p<0.001; OR 6.31 (95% CI 2.274 to 17.510)).

The effect of ADP-MPA on ischaemic events in different CYP2C19 genotype metabolisers was analysed (figure 3). ADP-MPA could predict ischaemic events in FMs (p=0.004, AUC 0.850 (95% CI 0.714 to 0.987)), the cut-off value was ADP-MPA=43.0% (sensitivity, 83.3%; specificity, 82.6%), and in IMs (p=0.003, AUC 0.757 (95% CI 0.650 to 0.864)), the cut-off value was ADP-MPA=44.3% (sensitivity, 83.3%; specificity, 66.2%). In UMs and PMs, ADP-MPA could not predict ischaemic events.

The patients were divided into a flow diverter group and a traditional stent group according to the type of stent used. The ischaemic event rates were 7.0% (14 of 199) in the flow diverter group and 4.4% (8 of 180) in the traditional stent group. Although the incidence of ischaemic events was higher in the flow diverter group than in the traditional stent group, the difference was not significant (p=0.285). ROC analysis demonstrated that ADP-MPA was predictive of ischaemic events in the flow diverter group (p=0.001; AUC 0.755 (95% CI 0.659 to 0.851); figure 4), with a cut-off value of 36.4% (sensitivity, 92.9%; specificity, 52.4%). ADP-MPA was also predictive of ischaemic events in the traditional stent group (p=0.006; AUC 0.786 (95% CI 0.644 to 0.929); figure 4), with a cut-off value of 42.9% (sensitivity, 87.5%; specificity, 68.6%).

DISCUSSION

Principal findings

In this study, we found that under standard DAT, CYP2C19 loss-of-function alleles led to a decreased antiplatelet aggregation effect of clopidogrel, but did not necessarily lead to HTPR. In addition to CYP2C19 loss-of-function genotypes, other factors that led to reduced antiplatelet aggregation function included age, hypertension

Table 2 Relevant factors for ADP-MPA

Variables	Unstandardised coefficient	SE	Standardisation coefficient	T value	P value
Constant term	9.269	4.821		1.923	0.055
Age	0.260	0.065	0.195	3.992	<0.001
Hypertension	3.108	1.357	0.109	2.290	0.023
Platelet count	0.025	0.011	0.109	2.311	0.021
IMs	7.622	1.415	0.271	5.388	<0.001
PMs	12.911	2.071	0.312	6.235	<0.001

ADP-MPA, maximum platelet aggregation rate induced by ADP; IMs, intermediate metabolisers; PMs, poor metabolisers.

Table 3 Predictors for ischaemic events

	Univariate logistic regression analysis		
Variables	**P value**	**OR**	**95% CI**
Female	0.062	0.438	0.184 to 1.043
Age	0.146	1.034	0.988 to 1.082
Smoking	0.246	1.784	0.671 to 4.742
Drinking	0.130	2.422	0.770 to 7.620
Hypertension duration >10 years	0.005	3.250	1.361 to 7.763
Hyperlipidaemia	0.825	1.153	0.327 to 4.059
Diabetes	0.028	3.058	1.130 to 8.274
Coronary artery disease	0.795	0.762	0.098 to 5.942
Cerebrovascular stenosis	0.201	1.894	0.712 to 5.041
Ischaemic cerebrovascular disease	0.409	1.855	0.517 to 6.655
Red cell count	0.213	1.614	0.760 to 3.425
White cell count	0.986	1.000	0.985 to 1.106
Platelet count	0.627	1.002	0.995 to 1.008
APTT	0.748	0.978	0.855 to 1.119
INR	0.364	3.940	0.204 to 76.083
Posterior circulation UIA	0.007	3.509	1.400 to 8.792
Maximum size	0.320	1.032	0.969 to 1.100
Multiple aneurysms	0.426	1.484	0.561 to 3.928
Non-saccular aneurysms	0.082	2.544	0.887 to 7.295
Flow diverters	0.285	1.627	0.666 to 3.974
LTA			
ADP-MPA	<0.001	1.066	1.031 to 1.102
AA-MPA	0.094	1.028	0995 to 1.063
Metabolic types			
Fast metabolisers	1	1	1
Ultra-fast metabolisers	0.999	–	–
Intermediate metabolisers	0.212	1.898	0.695 to 5.187
Poor metabolisers (%)	0.249	2.159	0.584 to 7.984

AA-MPA, maximum platelet aggregation rate induced by arachidonic acid; ADP-MPA, maximum platelet aggregation rate induced by ADP; APTT, activated partial thromboplastin time; INR, international normalised ratio; LTA, light transmission aggregometry; UIA, unruptured intracranial aneurysm.

and platelet count. For patients with UIAs treated with intracranial stent neurointerventional therapy, preoperative ADP-MPA was associated with the occurrence of ischaemic events, and ADP-MPA ≥41.5% could predict the risk of ischaemic events. Patients with ADP-MPA ≥41.5% had a >5-fold higher risk of ischaemic complications than patients with ADP-MPA <41.5%. This study further identified hypertension duration >10 years as an independent risk factor for ischaemic events in patients with selective UIA neurointervention. ADP-MPA could predict ischaemic events in FMs and IMs. The cut-off value of ADP-MPA for the occurrence of ischaemic events in patients with flow diverters was lower than in patients with traditional stents.

Table 4 Risk factors for ischaemic events

	Multivariate logical regression analysis		
Variables	**P value**	**OR**	**95% CI**
Hypertension duration >10 years	0.022	3.009	1.175 to 7.709
ADP-MPA	0.001	1.073	1.029 to 1.119

ADP-MPA, maximum platelet aggregation rate induced by ADP.

LTA and VerifyNow

Current methods commonly used for platelet function monitoring include LTA and VerifyNow (Accumetrics, San Diego, California, USA). VerifyNow is widely used worldwide because of its rapid performance and ease of use, and many studies have reported optimal cut-off values for predicting ischaemic and bleeding events in clopidogrel users.[19–21] Based on these reports, the normal reference range recommended by VerifyNow is 60–240 P2Y12 reaction units,[22] and antiplatelet regimens guided by VerifyNow results can reduce ischaemic events and bleeding events in interventional therapy.[23 24] As the classic method for monitoring platelet aggregation function, LTA is considered the gold standard, and is widely used because of its low cost. Although previous studies confirmed that LTA can predict ischaemic events and can be used to guide the adjustment of antiplatelet regimens,[13 25] it is important to establish a cut-off value for HTPR based on LTA. The observed indexes on LTA

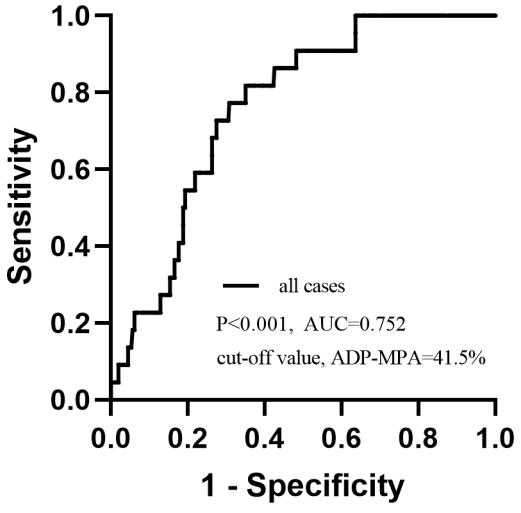

Figure 2 Receiver operating characteristic curve analysis of ischaemic events in all cases. ADP-MPA, maximum platelet aggregation rate induced by ADP; AUC, area under the curve.

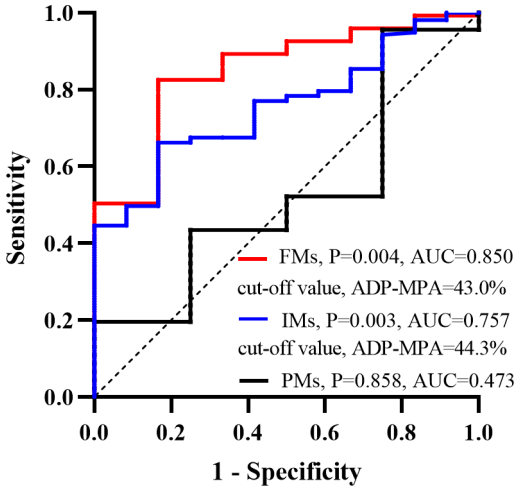

Figure 3 Receiver operating characteristic curve analysis of ischaemic events in different CYP2C19 metabolisers. ADP-MPA, maximum platelet aggregation rate induced by ADP; AUC, area under the curve; FMs, fast metabolisers; IMs, intermediate metabolisers; PMs, poor metabolisers.

include ADP-MPA, AA-MPA and COL-MPA. COL-MPA refers to the MPA rate induced by collagen and is usually employed as an auxiliary indicator for ADP-MPA and AA-MPA to judge bleeding tendency. Although AA-MPA was used to assess reactivity to aspirin, this study showed that a very small proportion of patients were resistant to aspirin (2.9%) and further demonstrated that AA-MPA was not associated with ischaemic events. Therefore, ADP-MPA was primarily used in the present study.

ADP-MPA predicts ischaemic events

Previous studies tried to determine optimal cut-off values for HTPR. In the cardiovascular literature, Paniccia *et al*[26] reported that the optimal cut-off value for HTPR based on ADP-MPA at 2 µmol/L and 10 µmol/L was 54% and 66%, respectively, while Tantry *et al*[14] reported that the optimal cut-off value was 46%. In the neurointervention literature, Adeeb *et al*[19] investigated 95 patients with intracranial aneurysms treated with flow diverter implantation, of which 4 had thromboembolic complications, and found that ADP-MPA=50% could distinguish between thrombotic and non-thrombotic events. Kan *et al*[25] examined 132 patients with intracranial aneurysms treated with stent-assisted coils, including 5 patients with thromboembolic complications, and reported that a cut-off value of 62% could predict thrombotic events. In the present study, the optimal cut-off value for ADP-MPA was 41.5%, which is significantly lower than the values reported in the above studies. When we grouped patients according to this cut-off value, we found that patients with ADP-MPA ≥41.5% had a >5-fold greater risk of ischaemic complications than patients with ADP-MPA <41.5%, indicating that the cut-off value for HTPR in the above literature could be larger.

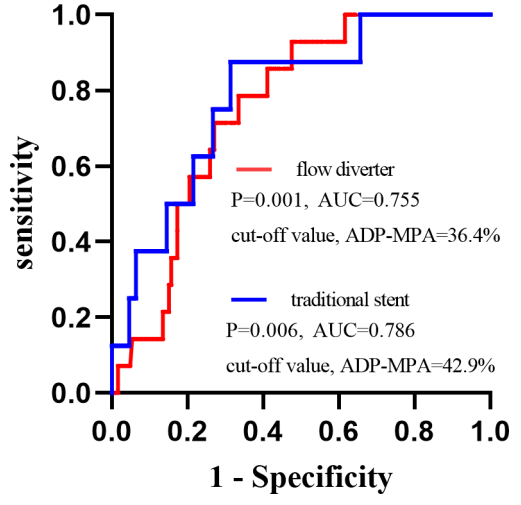

Figure 4 Receiver operating characteristic curve analysis of ischaemic events in patients with flow diverters and patients with traditional stents. Red line, flow diverter group; blue line, traditional stent group. ADP-MPA, maximum platelet aggregation rate induced by ADP; AUC, area under the curve.

Flow diverters and ischaemic events

The main difference between flow diverters and traditional stents is the high metal coverage of flow diverters. After implantation into a blood vessel, the main risk is that flow diverters could lead to thrombosis formation in the blood vessel and consequently cause ischaemic complications. Therefore, patients with flow diverters should take antiplatelet drugs for a longer time after the operation than patients with traditional stents. In patients with antiplatelet drug resistance, the risk of ischaemic complications could be increased. The results of the present study suggest that the use of flow diverters has a higher risk of ischaemic events than the use of traditional stents (7.0% vs 4.4%). Furthermore, when ADP-MPA was used to predict ischaemic events, the cut-off value in the flow diverter group was lower than in the traditional stent group (36.4% vs 42.9%), suggesting that flow diverters had a greater tendency to cause thrombotic events under the same platelet aggregation function. Therefore, in patients with flow diverters, it is unsafe to use the same antiplatelet regimen used in patients with traditional stents, and a stronger antiplatelet regimen could be needed before stent placement, especially in patients with ADP-MPA test results above the cut-off value.

Effect of CYP2C19 genotypes

CYP2C19 genotype testing is another method of assessing patient response to clopidogrel. Our comparison of the LTA and CYP2C19 genotype test results showed that if LTA indicated HTPR, patients were highly likely to carry CYP2C19 loss-of-function alleles, although carrying CYP2C19 loss-of-function alleles was not a decisive factor for HTPR. Many studies have investigated the relationships between CYP2C19 genotypes and ischaemic complications. The meta-analysis conducted by Pan *et al*[15] showed that carriers of CYP2C19 loss-of-function alleles had a greater risk of stroke and composite vascular events

than non-carriers among patients with ischaemic stroke or TIA treated with clopidogrel, while Ge *et al*[27] found that patients with stent-assisted coil therapy for intracranial aneurysms who carried CYP2C19 loss-of-function alleles had increased ischaemic events under standard DAT. However, there was no significant association between CYP2C19 loss-of-function alleles and ischaemic complications in this study, although IMs and PMs were positively correlated with ADP-MPA. Compared with the meta-analysis by Pan *et al*, differences in the target patients, treatment measures and sample sizes could account for the different results. In the study by Ge *et al*, a broader term for ischaemic complications was used, and the proportion of ischaemic events reached 32.1% because they included both patients with clinical symptoms and asymptomatic patients with imaging evidence.

Several factors could contribute to why CYP2C19 genotypes were unrelated to ischaemic events. First, CYP2C19 is not the only enzyme involved in the metabolic process for clopidogrel,[8] and ABCB1 genetic variants can also affect clopidogrel metabolism.[18] Second, according to the multiple linear regression results, platelet function was also affected by age, hypertension and platelet count, and previous studies showed that chronic diseases such as diabetes and renal insufficiency are other important causes of HTPR. Third, clinical factors such as hypertension were identified as independent risk factors for ischaemic complications. Finally, in neurointerventional therapy, operational experience and neurointerventional instruments are important factors that can lead to ischaemic complications. Overall, our results suggest that LTA can be recommended over CYP2C19 genotype testing for neurointerventional procedures in patients with UIA, which is consistent with the recommendations of the American Heart Association and the American College of Cardiology Foundation against routine CYP2C19 gene testing for all patients.[28]

Factors influencing platelet aggregation

In previous studies, clopidogrel hyporesponsiveness was associated with female sex, age, high body weight, diabetes and chronic kidney disease.[7 10 29 30] In the present study, platelet count, age and hypertension were positively and independently correlated with ADP-MPA. Because platelet count reflects the basis of platelet aggregation, it is easy to understand and physiologically consistent that an increased number of platelets will lead to more intense platelet aggregation, and Lee *et al*[29] reported a similar result. We also found that age was an important factor affecting platelet aggregation, given that ADP-MPA increased by 0.26% for each year increase in age. The likely reason is that as age increases, the metabolic activity of the cytochrome P450 enzyme decreases, which affects clopidogrel activation. In addition, elderly people with chronic diseases take more drugs; therefore, drug interactions could also inhibit clopidogrel activation. For example, Catapano *et al*[9] reported that omeprazole was associated with a significant decrease in the antiplatelet aggregation function of clopidogrel. In the present study, we found for the first time that hypertension affects platelet reactivity. Specifically, patients with hypertension had 3.14% higher ADP-MPA on average than patients without hypertension. In addition to the drug interactions described above, other reasons could be that hypertension acts on the vascular wall and causes endothelial cell damage, thereby promoting platelet aggregation.

Limitations

The first and most significant limitation of this study was its retrospective design, which means it could have incomplete data, recall bias and unknown confounding factors. Second, the study was conducted in a single institution because LTA testing lacks uniform standards among institutions, thus limiting the universality of the results. However, this study provides an important reference for institutions that use LTA as a primary monitoring tool. Third, the strict screening criteria excluded a large proportion of patients from the analysis, particularly patients who underwent antiplatelet adjustment, which could have resulted in a deviation between the study results and the actual results. Finally, the study only examined clinically symptomatic strokes and did not analyse asymptomatic radiographic strokes.

CONCLUSIONS

Our results suggest that carrying CYP2C19 loss-of-function alleles is an important cause of HTPR, but is not related to ischaemic events. ADP-MPA can predict ischaemic events, and the determined cut-off values indicate that patients with flow diverters require a stronger antiplatelet regimen than patients with traditional stents. This study could provide a promising method for adjusting appropriate antiplatelet therapy.

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