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ORIGINAL RESEARCH

Impact of Platelet Hyperreactivity and Diabetes Mellitus on Ischemic Stroke Recurrence: A Single-Center Cohort Clinical Study

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Purpose: Ischemic stroke recurrence (ISR) is prevented by inhibiting platelet function. To investigate the impact of high on-treatment platelet reactivity (HTPR) assessed by thromboelastography (TEG) and its risk factors on ISR in individuals who have experienced acute ischemic stroke (AIS) receiving dual anti-platelet therapy (DAPT).

Patients and Methods: At the end of follow-up, a total of 264 patients who met the criteria were enrolled in this cohort study. The primary endpoint event was a recurrence of ischemic stroke within 90 days of onset.

Results: The ISR rate was 7.2% (19/264). The recurrence rate in the HTPR group was 15.1% (8/53), which was significantly higher than the 5.2% (11/211) in the non-HTPR group (p = 0.013), and the type 2 diabetes mellitus (T2DM) group (12.5%, 10/80) was also significantly higher compared to the non-T2DM group (4.9%, 9/184) (p = 0.028). T2DM was an isolated risk factor for HTPR (adjusted OR = 3.06, 95% CI 1.57–5.98, P = 0.001). Kaplan-Meier plots showed that the cumulative risk (CR) of ISR was statistically different in the HTPR and T2DM groups compared to the non-HTPR group (log-rank P = 0.009) and the non-T2DM group (log-rank P = 0.026), respectively. The HTPR and T2DM groups had greater hazard ratios (HR) of ISR than the non-HTPR (adjusted HR = 2.78, 95% CI 1.06–7.32, P = 0.038) and non-T2DM (adjusted HR = 2.64, 95% CI 1.01–6.92, P = 0.049) groups.

Conclusion: Both HTPR and T2DM are linked to ISR. Platelet Inhibition Rate (PIR) of TEG can early identify patients who are at high risk for having another ischemic stroke in patients undergoing DAPT, and this study may offer more evidence in favor of clinically personalized treatment and secondary prevention tactics.

Keywords: ischemic stroke, diabetes mellitus, clopidogrel, thrombelastography, platelet activation

Introduction

Ischemic stroke recurrence (ISR) is linked to numerous factors,[1,2] including poor antithrombotic effects. The guidelines recommend that patients with noncardioembolic mild ischemic stroke be treated with dual antiplatelet therapy (DAPT) with aspirin and clopidogrel,[3–5] which significantly reduces the rate of ISR. However, studies have shown that ischemic recurrence still occurs in 5–10% of stroke patients in the first three months after receiving DAPT,[6–8] which may be related to platelet reactivity.

Laboratory measurements of platelet function below the cut-off value are defined as high on-treatment platelet reactivity (HTPR), which reflects the poor effect of anti-platelet therapy due to individualized differences. According to earlier research in individuals who received DAPT for peripheral arterial disease and coronary artery disease, HTPR is assumed to be brought on by clopidogrel's insufficient platelet inhibition.[9,10] Clopidogrel, a prodrug, is the most widely used P2Y12 receptor antagonist and is bio-transformed in vivo into an active metabolite that irreversibly binds to the platelet adenosine diphosphate (ADP) receptor P2Y12, blocking ADP-induced activation of the GPIIb-IIIa complex and preventing the aggregation of platelets. Individualized differences in clopidogrel are influenced by a variety of factors.[11–13] Nonetheless, the precise mechanisms have not been completely elucidated. Platelet aggregation analyzers currently on

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the market for detecting the inhibitory effect of clopidogrel on platelet ADP receptors include light transmission aggregometry (LTA) using platelet-rich plasma and multiple electrode aggregometry (MEA), VerifyNow, PL-11/12, and platelet function analyzers (PFA-100/200) using whole blood.[14–17] In recent years, thromboelastography (TEG), which uses ADP-induced accumulation of platelets in vitro to diagnose HTPR to clopidogrel in the laboratory, has begun to be applied to assess the efficacy of clinical anti-thrombotic therapy. The advantage of the TEG over their sole function of detecting platelet aggregation is that it uses whole blood containing clotting factors, platelets, and fibrinogen, which are essential for coagulation, and adds an aggregating agent to mimic in vitro the entire in vivo coagulation-triggered cascade of clotting reactions.

However, previous studies have focused almost exclusively on risk factors for HTPR,[18,19] and few have examined the impact of HTPR and its associated risk factors on ISR. In addition, it is unclear whether TEG can identify HTPR reliably and predict ISR early while patients with acute ischemic stroke (AIS) undergo DAPT. In the present investigation, we determined that both HTPR and T2DM are associated with ISR. The clinical use of TEG in ischemic stroke will offer more evidence in favor of clinically personalized treatment and secondary prevention tactics.

Materials and Methods

Study Design and Population

This was a single-center, observational cohort study that comprised 264 Chinese Han AIS patients on DAPT from July 2020 to December 2021. Of these patients, 232 had their first ischemic stroke, and the remaining 32 had a history of previous ischemic strokes. We ended the follow-up with all patients in March 2022. Inclusion criteria for this study included: 18 years of age or older; minor ischemic stroke within 24 hours of onset (score of the National Institutes of Health Stroke Scale $\leq$ 3); diagnosis of AIS based on clinical symptoms and imaging techniques such as magnetic resonance imaging or computed tomography; and at least three days of combination therapy with clopidogrel (75 mg/day; first day loading dose of 300mg) and aspirin (100 mg/day) prior to platelet function testing. Exclusion criteria: ischemic stroke due to cardiogenic embolism based on an electrocardiogram; Other causes of central nervous system (CNS) disorders such as vascular malformations and tumors; Intravenous thrombolysis, anticoagulation and endovascular therapy; previous history of ischemic stroke treated with non-aspirin plus clopidogrel antiplatelet therapy; severe hepatic and renal insufficiency; history of malignancy, rheumatic immune disease, and blood disorders related to coagulation dysfunction; contraindications to clopidogrel and aspirin; hemorrhagic transformation after an initial ischemic stroke based on imaging techniques such as magnetic resonance imaging or computed tomography; failure to comply with medical advice on secondary prevention during follow-up visits; missing patients. Neurologists completed the inclusion and exclusion criteria. The present study received ethical approval from the Ethics Committee of the First Affiliated Hospital of Anhui Medical University.

Blood Specimen Collection

After receiving DAPT for a minimum of 3 days, at least 2 mL of whole blood was collected from the peripheral vein using vacuum blood collection tubes containing 3.2% sodium citrate and heparinization (concentration should be >14.5 IU heparin/mL), respectively. These samples were gently turned up and down five times after blood collection to allow the blood to mix well with the anticoagulant in the collection tube until it was completely anticoagulated, and then they were immediately sent to the transfusion department of the hospital.

Platelet Inhibition Rate Assay

All analyses were performed according to the manufacturer's standard operating procedures, and the Platelet Inhibition Rate (PIR) was measured using a TEG hemostasis analyzer (Haemonetics Corporation, USA) within 2 hours of collecting the blood samples. The effect of anti-platelet therapy in patients was tested by activation of uninhibited platelet receptors by arachidonic acid (AA) and adenosine diphosphate (ADP) at a temperature of 37◦C. This process takes four cups to complete. The detection steps were as follows: 1. Load the sample cup. 2. Prepare the reagents as required. 3. Add 1 mL of well-mixed sodium citrate anticoagulated whole blood to Reagent R, cap the bottle, and gently

turn it up and down to shake it well. Add 20 μL of 0.2M calcium chloride solution to the sample cup, and then add 340 μL of the mixed blood to the sample cup. 4. Immediately push the cup holder up and turn the lever to the test position. 5. Click the Start button on the toolbar of the software to test. 6. Start running the sample until the MA value was determined. 7. Pipette 10 μL of fully dissolved A (activator F) reagent into a blank sample cup, then pipette 360 μL of well-mixed, heparinized whole blood into the sample cup with A reagent, and repeatedly pipette the blood into the sample cup three times to mix it. 8. Repeat steps 4–6 for MAF values. 9. Pipette 10 μL of A reagent and ADP reagent into a blank sample cup, respectively, and then pipette 360 μL of well-mixed, heparinized whole blood into the sample cup spiked with A reagent and ADP reagent, and repeatedly pipette the blood in the sample cup three times to make the blood mix. 10. Repeat steps 4–6 for MAADP value detection. 11.Pipet 10 μL of A reagent and AA reagent into a blank sample cup, respectively, then pipet 360 μL of well-mixed, heparinized whole blood into the sample cup spiked with A reagent and AA reagent, and repeatedly pipet the blood in the sample cup three times to make the mixture. 12. Repeat steps 4–6 for MAAA value detection. MA is the maximum amplitude on the TEG, reflecting the greatest strength and stability of the clot being formed. MAADP represents the clot strength induced by ADP. MAAA represents the strength of the clot induced by AA, and MAF represents clot strength with fibrin only. The computer program was used to calculate the PIR caused by AA or ADP, and ADP-induced PIR (%) = 100%-(MAADP-MAF)/(MA-MAF)*100%, representing the responsiveness to clopidogrel on platelets. An ADP-induced PIR (%) of less than 30% was defined as HTPR after the clopidogrel maintenance dose reached a steady state (implying a state of maximal platelet aggregation inhibition), whereas the PIR in patients with non-HTPR was at least 30% according to the instructions and previous studies.[20]

Data Quality Control

Cross-checking of data after data entry by two specially trained physicians; secondary prevention education for patients and their families during hospitalization; and follow-up visits were made by telephone or in the outpatient clinic after discharge to ensure adherence to secondary prevention.

Outcomes

All patients received telephone or outpatient follow-up visits by trained investigators within 3 months. The primary outcome was an ISR. ISR was defined as the development of new ipsilateral or contralateral signs and symptoms of focal or diffuse neurological deficits after improvement of preexisting CNS signs and symptoms and the formation of new ipsilateral or contralateral focal cerebral infarct foci on imaging techniques such as magnetic resonance imaging or computed tomography scanning, which were confirmed to be nonhemorrhagic or noncardiac in origin.

Statistical Analysis

We used IBM SPSS 22.0 software (IBM Corporation, Armonk, NY, USA) to conduct the statistical analyses. The frequencies and percentages were used to represent variables that are categorical, while mean ± standard deviation (SD) or median [interquartile range] were used to express variables that are continuous. The normal distribution test was performed using the Shapiro–Wilk test. Comparisons across groups were conducted utilizing two-sided t-tests, the Mann–Whitney *U*-test, and the Pearson chi-square test, as appropriate for the specific distribution. Stepwise univariate analysis, univariate logistic regression analysis, and multivariate logistic regression analysis were used to assess the risk factors for HTPR. A Pearson chi-square test analysis was conducted to compare the prevalence of HTPR between patients in the T2DM and non-T2DM groups, the recurrence rate of ischemic stroke between patients in the HTPR and non-HTPR groups, and the recurrence rate of ischemic stroke between patients in the T2DM and non-T2DM groups. Using the Kaplan-Meier method, we compared the cumulative risk (CR) of ISR between T2DM and non-T2DM patients, as well as between HTPR and non-HTPR patients. Subsequently, we constructed Cox proportional risk regression models to calculate HR for the primary endpoint between the HTPR and non-HTPR groups and between the T2DM and non-T2DM groups. The forest plot displays the HR obtained using Cox proportional risk regression models, comparing the T2DM and non-T2DM groups, as well as the HTPR and non-HTPR groups. Statistical significance was determined by using two-tailed p-values less than 0.05.

Results

Demographic and Clinical Characteristics

We included 264 individuals with AIS who underwent DAPT in this study (Figure 1). According to the defined HTPR, individuals were categorized into two cohorts: the HTPR (53, 20.1%) group and the non-HTPR (211, 79.9%) group. Gender (male), T2DM, neutrophil count, monocyte count, red blood cell count, hemoglobin, platelet count, alkaline phosphatase, apolipoprotein B, and glucose were statistically significant in both groups, while other parameters were not statistically significant. Table 1 shows the demographic and clinical characteristics of the patients.

Risk Factors for HTPR

The risk variables associated with HTPR are shown in Table 2. Univariate logistic regression analysis: we used the HTPR grouping as the dependent variable, and the parameters that were statistically significant for univariate analysis in Table 1 were placed in the independent variable column one by one. The analysis showed that gender (male), T2DM, neutrophil count, monocyte count, red blood cell count, and hemoglobin were statistically significant. Multivariate logistic regression analysis: HTPR grouping was used as the dependent variable, and all statistically significant parameters from

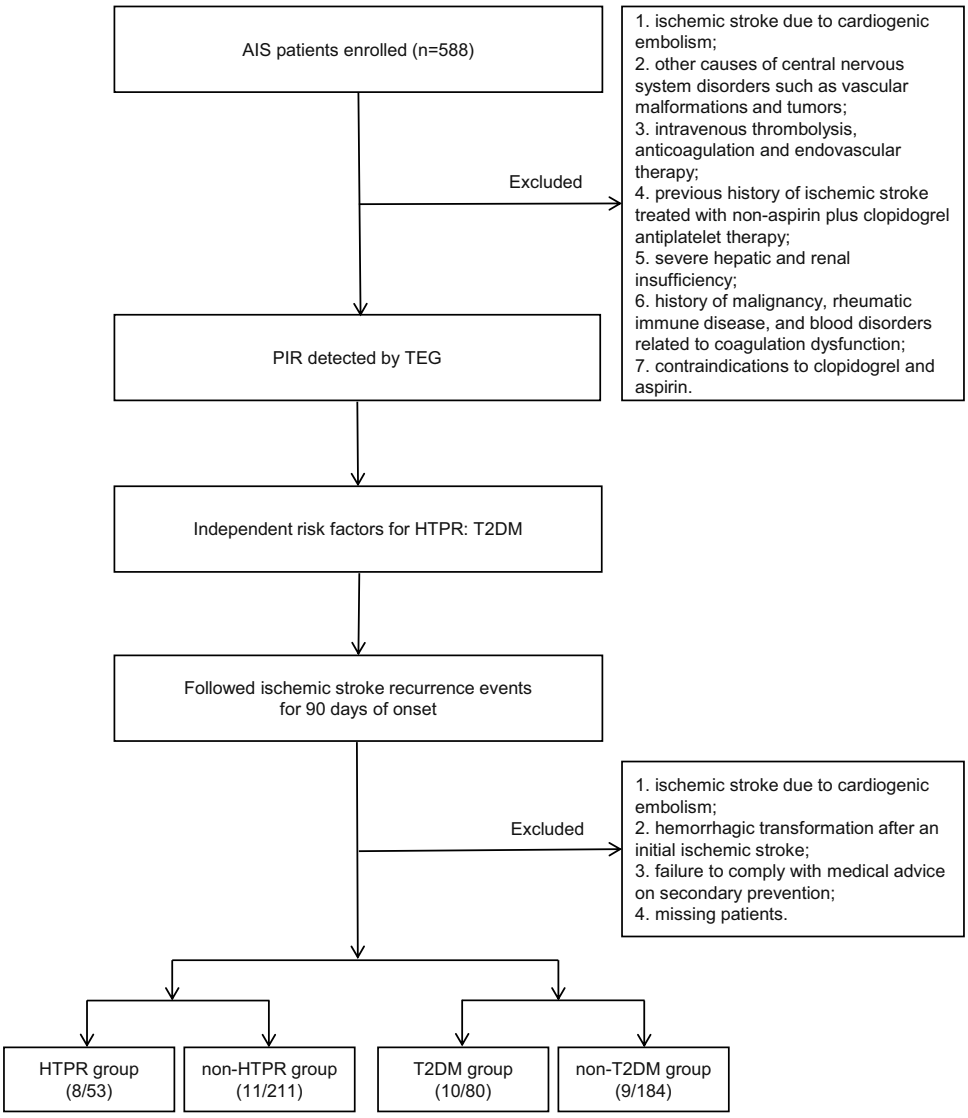

Figure 1 Flowchart of patient recruitment.

Table 1 Demographic and Clinical Characteristics of the Patients

Characteristics	HTPR(n=53)	non-HTPR(n=211)	P-value
Age (y)	64(56.5–73.0)	62(54.0–70.0)	0.187
Male. n (%)	35(66.0)	172(81.5)	**0.014**
Smoking. n (%)	7(13.2)	46(21.8)	0.163
T2DM. n (%)	27(50.9)	53(25.1)	**<0.001**
Medical history. n (%)			
Previous ischemic stroke	10(18.9)	22(10.4)	0.092
Hypertension	35(66.0)	143(67.8)	0.810
Smoking	12(22.6)	66(31.3)	0.218
Multiple cerebral infarction	21(39.6)	77(36.5)	0.673
Laboratory data			
RBC (*10^{12}/L)	4.3±0.5	4.5±0.5	**0.016**
HGB (g/L)	133.1±16.9	138.6±14.3	**0.016**
NEUT (*10^{9}/L)	3.45(2.81–4.51)	4.25(3.40–5.31)	**0.001**
MONO (*10^{9}/L)	0.36(0.27–0.46)	0.42(0.34–0.50)	**0.001**
PLT (*10^{9}/L)	235(187–294)	214(173–260)	**0.049**
PCT (%)	0.25(0.21–0.30)	0.23(0.19–0.28)	0.093
PDW (f l)	12.4(11.3–14.4)	12.8(11.6–14.8)	0.395
MPV (f l)	10.6(10.1–11.5)	10.8(10.2–11.6)	0.322
ALP (U/L)	68(63–82)	78(65–91)	**0.029**
LDL-C (mmol/L)	2.05(1.54–2.53)	1.79(1.37–2.29)	0.066
GGT (U/L)	22.5(16.3–43.3)	26.0(17.0–42.0)	0.544
LDH (U/L)	172.5(152.5–189.0)	170.0(151.0–194.0)	0.992
eGFR (mL.1.73m*2)	99.0(83.5–110.5)	101.0(90.0–110.0)	0.460
TC (mmol/L)	3.61(3.04–4.41)	3.35(2.90–3.98)	0.114
TG (mmol/L)	1.36(0.93–1.80)	1.32(0.97–1.83)	0.831
HDL-C (mmol/L)	0.98(0.85–1.20)	0.95(0.84–1.11)	0.324
n-HDL (mmol/L)	2.61(1.92–3.27)	2.32(1.92–2.94)	0.112
VLDL (mmol/L)	0.50(0.34–0.67)	0.49(0.36–0.68)	0.741
APOA (g/L)	1.10(0.91–1.22)	1.03(0.91–1.18)	0.375
APOB (g/L)	0.81(0.65–0.91)	0.72(0.60–0.84)	**0.035**
GLU (mmol/L)	5.62(5.06–7.19)	5.33(4.89–6.35)	**0.049**

Notes: Data are expressed as mean ± SD, median (25th and 75th percentiles), or percentage (frequency). The significance of the comparison was determined by a *t*-test, a Mann–Whitney *U*-test, or a Pearson chi-square test. Two-tailed P values <0.05 were considered statistically significant. Bold values indicate statistical significance.

Abbreviations: RBC, red blood cell count; HGB, hemoglobin; NEUT, neutrophil count; MONO, monocyte count; PLT, platelet count; PCT, platelet hematocrit; PDW, platelet distribution width; MPV, mean platelet volume; ALP, alkaline phosphatase; GGT, Y-glutamyl transferase; LDH, lactate dehydrogenase; eGFR, estimated glomerular filtration rate; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; n-HDL, non-HDL cholesterol; VLDL, very low-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; APOA, apolipoprotein A; APOB, apolipoprotein B; GLU, glucose.

univariate logistic regression analysis were put into the independent variable column. The findings of the research revealed that T2DM was an isolated risk factor for HTPR (adjusted OR = 3.06; 95% CI 1.57–5.98; P = 0.001).

T2DM and Coagulation Test

Table 3 presents the coagulation test parameters for individuals belonging to the non-T2DM and T2DM cohorts. Comparative analysis of TEG parameters between the two groups showed no statistical significance for parameters (R, K, Angle, MA, CI, MAA, MAAA, and AA-induced PIR), while MAADP (26.80 [15.63–39.65] vs 37.50 [19.50–51.48], P = 0.001) and ADP-induced PIR (65.95 [43.98–87.70] vs 47.70 [24.65–79.23], P = 0.004) were statistically significant. Comparative analysis of conventional coagulation test parameters between the two groups showed no statistical significance for parameters (FIB, TT, AT-III, D-D, and FDP), while PT (10.30 [9.88–10.83] vs 10.10 [9.70–10.60], P

Table 2 Risk Factors for HTPR

Variables	Univariate Logistic Regression			Multivariate Logistic Regression		
	P-value	OR	95% CI	P-value	OR	95% CI
Male. n (%)	**0.016**	2.27	1.17–4.42	0.165	0.57	0.25–1.27
T2DM. n (%)	**<0.001**	3.10	1.66–5.77	**0.001**	3.06	1.57–5.98
RBC (*10^{12}/L)	**0.017**	2.14	1.14–4.01	0.119	2.99	0.75–11.87
HGB (g/L)	**0.017**	1.03	1.00–1.05	0.422	0.98	0.94–1.03
NEUT (*10^9/L)	**0.003**	1.46	1.14–1.88	0.057	1.34	0.99–1.81
MONO (*10^9/L)	**0.002**	55.45	4.29–717.39	0.147	9.74	0.45–211.32
PLT (*10^9/L)	0.058	0.99	0.99–1.00	-	-	-
ALP (U/L)	0.084	1.01	1.00–1.03	-	-	-
APOB (g/L)	0.081	0.24	0.05–1.19	-	-	-
GLU (mmol/L)	0.217	0.92	0.80–1.05	-	-	-

Notes: Two-tailed P values <0.05 were considered statistically significant. Bold values indicate statistical significance."-" indicates that multivariate logistic regression analysis was not performed.

Table 3 T2DM and Coagulation Test

Variables	Non-T2DM(n=184)	T2DM(n=80)	P-value
R (min)	5.20(4.30–6.20)	5.00(4.23–5.90)	0.499
K (min)	1.70(1.40–2.10)	1.60(1.23–2.08)	0.328
Angle (deg)	66.20(60.80–69.88)	66.35(61.13–70.80)	0.401
MA (mm)	63.90(59.20–67.50)	64.80(60.55–68.63)	0.099
CI	1.00(−0.30–2.00)	1.25(−0.05–2.50)	0.264
MAA (mm)	8.05(5.43–12.90)	9.25(5.80–13.78)	0.207
MAADP (mm)	26.80(15.63–39.65)	37.50(19.50–51.48)	**0.001**
MAAA (mm)	26.80(11.95–41.20)	30.75(17.35–41.50)	0.161
AA-induced PIR (%)	70.90(44.25–95.05)	66.10(42.40–84.88)	0.308
ADP-induced PIR (%)	65.95(43.98–87.70)	47.70(24.65–79.23)	**0.004**
PT (sec)	10.30(9.88–10.83)	10.10(9.70–10.60)	**0.025**
PT-INR	0.86(0.83–0.90)	0.84(0.81–0.88)	**0.029**
APTT (sec)	26.20(25.20–27.60)	25.30(24.60–26.60)	**<0.001**
FIB (g/L)	3.23(2.73–3.85)	3.28(2.76–4.17)	0.412
TT (sec)	17.90(17.48–18.43)	17.80(17.10–18.70)	0.347
AT-III (sec)	93.70(85.80–102.80)	96.80(89.70–102.00)	0.336
D-D (ug/L)	0.51(0.41–0.66)	0.59(0.45–0.73)	0.050
FDP (ug/L)	1.00(0.70–1.40)	1.10(0.70–1.80)	0.358

Notes: Data are expressed as medians (25th and 75th percentiles). The significance of comparisons was determined by the Mann–Whitney *U*-test. Two-tailed P values <0.05 were considered statistically significant. Bold values indicate statistical significance.

Abbreviations: AA-induced PIR (%), arachidonic acid-induced platelet inhibition rate (%); ADP-induced PIR (%), adenosine diphosphate-induced platelet inhibition rate (%); PT, plasma prothrombin time; PT-INR, plasma prothrombin time international normalized ratio; APTT, activated partial thromboplastin time; FIB, fibrinogen content; TT, plasma prothrombin time measurement; AT-III, antithrombin-III; D-D, D-dimer; FDP, fibrin (pro)degradation product.

= 0.025), PT-INR (0.86 [0.83–0.90] vs 0.84 [0.81–0.88], P = 0.029), and APTT (26.20 [25.20–27.60] vs 25.30 [24.60–26.60], P<0.001) were statistically significant.

T2DM and HTPR

Figure 2 shows the prevalence of HTPR in the cohort with and without T2DM. The prevalence of HTPR in the cohort without T2DM was 14.1% (26/184). However, this prevalence was substantially elevated in the cohort with T2DM, at

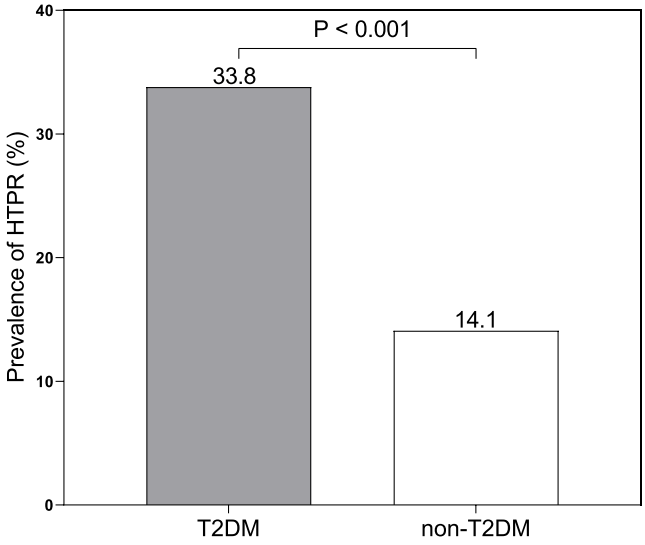

Figure 2 Comparison of the prevalence of HTPR between T2DM and non-T2DM groups. A Pearson chi-square test was used to determine the difference.

33.8% (27/80). The statistical analysis, using the Pearson chi-square test, indicated a substantial disparity in the occurrence of HTPR between the two cohorts (P<0.001, two-tailed).

Study Endpoints

The primary endpoint event was an ISR within 90 days of onset. During the follow-up, 19 of the 264 patients had an ISR. The recurrence rate (see Figure 3) in the HTPR group was 15.1% (8/53), which was significantly higher than the 5.2% (11/211) in the non-HTPR group (p = 0.013), and T2DM group (12.5%, 10/80) was also significantly higher compared to the non-T2DM group (4.9%, 9/184) (p = 0.028). Figure 4 shows the Kaplan-Meier curve constructed from HTPR and its risk factors (T2DM). In the HTPR cohort, the CR of ISR was 15.1% (8/53), while in the non-HTPR cohort, it was 5.2% (11/211) (log rank P = 0.009). Similarly, in the cohort with T2DM, the CR of ISR was 12.5% (10/80), while in the cohort without T2DM, it was 4.9% (9/184) (log rank P = 0.026). Figure 5 shows the HR forest plot of the impact of HTPR and T2DM on ischemic stroke recurrence. In the unadjusted Cox proportional risk regression model, the cohort with HTPR

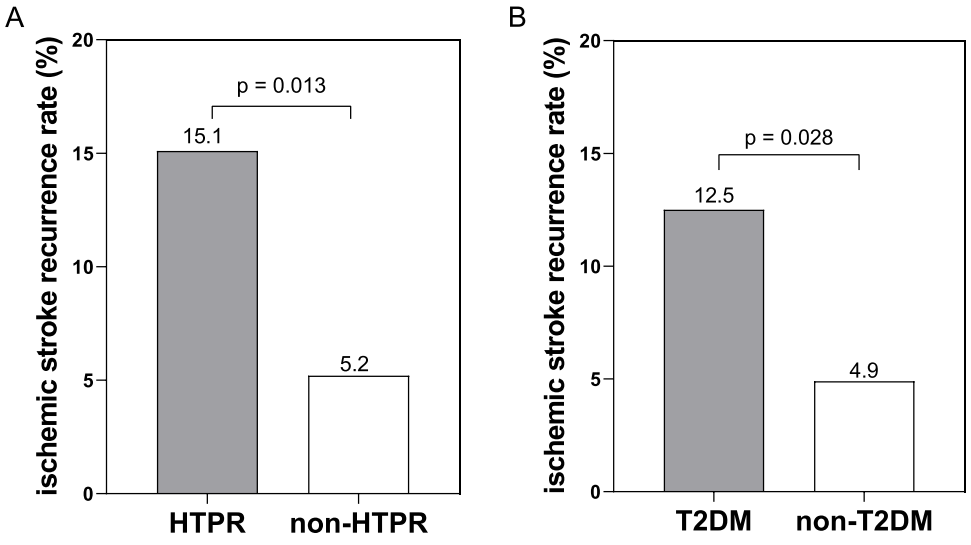

Figure 3 The recurrence rate of an ischemic stroke. (**A**) The Pearson chi-square test was used to determine the differences in ischemic stroke recurrence rates between patients in the HTPR group and the non-HTPR group; (**B**) The Pearson chi-square test was used to determine the differences in ischemic stroke recurrence rates between patients in the T2DM group and the non-T2DM group.

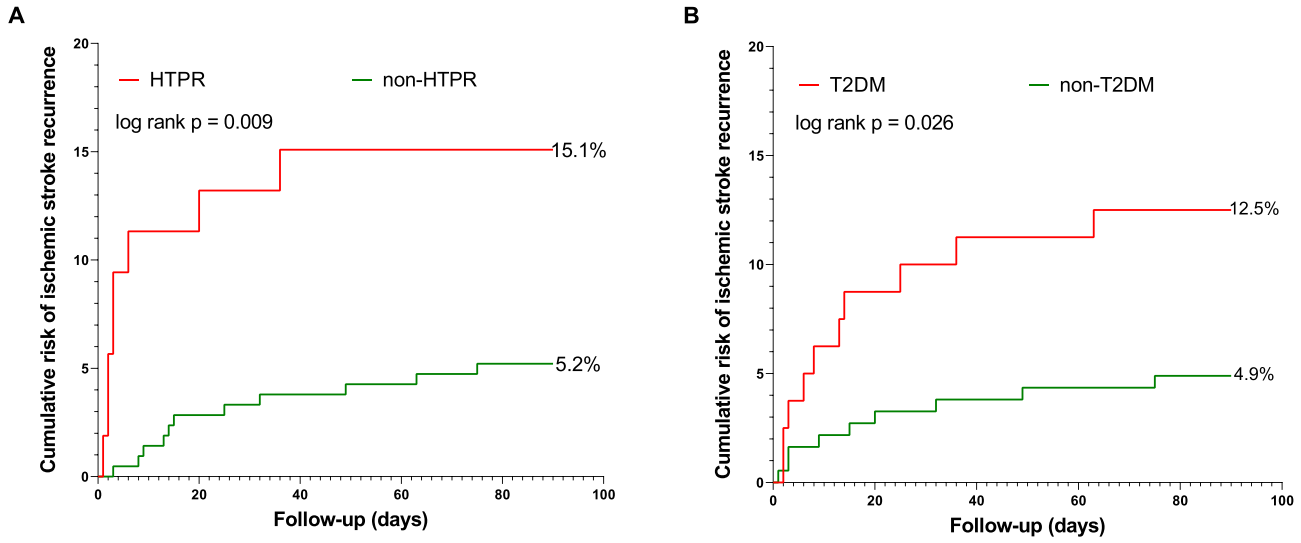

Figure 4 Kaplan-Meier curves constructed from HTPR and its risk factors (T2DM). (**A**) Kaplan-Meier curves depicting the cumulative risk of ischemic stroke recurrence in patients in the HTPR (ADP-induced PIR (%) <30%) group and non-HTPR (ADP-induced PIR (%) ≥30%) group; (**B**) Kaplan-Meier curves depicting the cumulative risk of ischemic stroke recurrence in patients in the T2DM group and non-T2DM group.

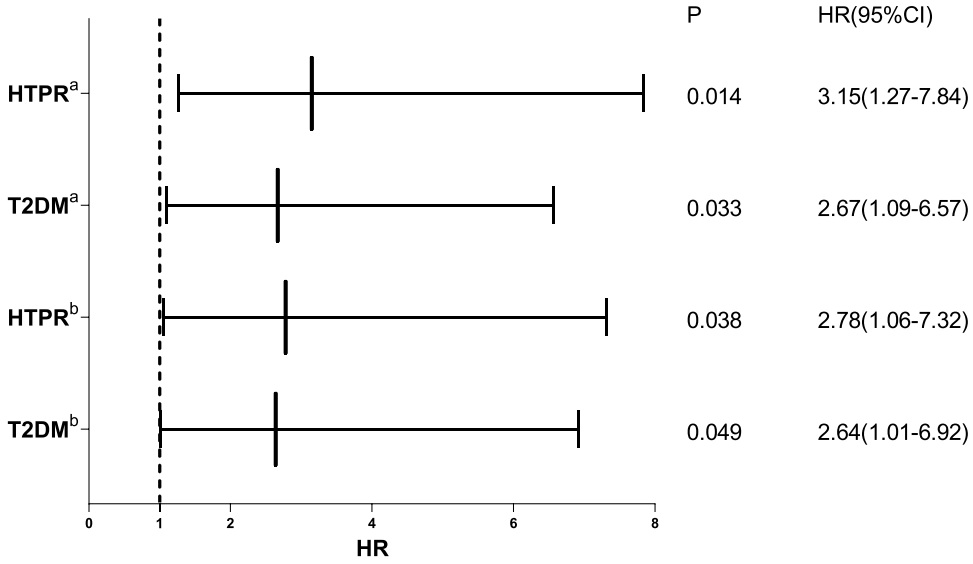

Figure 5 HR forest plot of ischemic stroke recurrence in the HTPR and its risk factors (T2DM) groups. [a]Unadjusted Cox proportional risk regression model; [b]adjusted Cox proportional risk regression model (age, sex, history of hypertension, history of smoking, current smoking, and multiple cerebral infarcts).

had a higher HR for ISR compared to the non-HTPR cohort (HR = 3.15, 95% CI 1.27–7.84, P = 0.014). Similarly, the cohort with T2DM showed a higher HR for ISR compared to the non-T2DM cohort (HR = 2.67, 95% CI 1.09–6.57, P = 0.033). In the adjusted Cox proportional risk regression model (age, gender, history of hypertension, history of smoking, current smoking, and multiple cerebral infarcts), the HR of ISR in the HTPR cohort remained markedly greater than in the non-HTPR cohort (adjusted HR = 2.78, 95% CI 1.06–7.32, P = 0.038). Similarly, the HR of ISR in the T2DM cohort remained markedly greater than in the non-T2DM cohort (adjusted HR = 2.64, 95% CI 1.01–6.92, P = 0.049).

Discussion

In our present study, we investigated whether HTPR and its risk factors studied by applying the TEG technique were associated with ISR. To clarify the effect of HTPR and its risk factors related to ISR, we measured PIR using the TEG technique and followed ISR events within 90 days of onset. Our results demonstrate that T2DM is a separate factor of

risk for HTPR, possibly caused by insufficient clopidogrel inhibition of activated platelets, and that both HTPR and T2DM are linked to ISR. Overall, TEG can identify HTPR reliably and predict ISR early, when patients with AIS undergo DAPT.

Previous studies have shown that the variability in the incidence of HTPR may be significant. According to a recent systematic review and meta-analysis,[21] in individuals with ischemic cerebrovascular disease, the prevalence of HTPR was 8–65% for Clopidogrel and only 1.8–35% for Clopidogrel plus aspirin. Fu et al evaluated the platelet function of clopidogrel-treated stroke patients using the LTA technique, and the prevalence of HTPR, which is defined as 5 uM ADP-induced platelet aggregation of more than 46%, was reported to be 48.1%.[22] Lundstrom et al studied platelet function (assessed by MEA technique) in stroke patients taking clopidogrel and found that the prevalence of HTPR (defined as an MEA ADP value greater than 468 AU*min) was 21.2%.[18] Rath et al studied platelet function (assessed by the VerifyNow technique) in stroke patients taking clopidogrel and found that the prevalence of HTPR (defined as patients who meet the drug requirements with PRU values exceeding 208) was 28.8%.[23] Similarly, our investigation showed that the prevalence of HTPR among individuals treated with DAPT was 20.1%. We consider that the variable prevalence of HTPR is related to the anti-platelet therapy drugs, the test method used to detect platelet reactivity, and the cut-off value that defines HTPR.

T2DM is an isolated risk factor for HTPR with clopidogrel. Previous studies have demonstrated that stroke individuals with DM have a 1.48 to 2.42 times higher risk of HTPR compared to stroke patients without DM.[24–26] Compared to their investigations, ours examines the impact of the diabetic subtype (T2DM) on clopidogrel's anti-thrombotic effect. The present investigation revealed that the likelihood of HTPR in the cohort with T2DM was 3.06 times greater compared to the group without T2DM. Those with T2DM had a lower ADP-induced PIR than those without T2DM (47.70 [24.65–79.23] vs 65.95 [43.98–87.70], P = 004). The prevalence of HTPR in the T2DM cohort was 33.8%, while the prevalence of HTPR in the non-T2DM cohort was only 14.1%.

TEG can predict ISR early in AIS patients receiving DAPT. The ischemic stroke recurrence rate in patients within 3 months of our follow-up was 7.2%, which is similar to the 5–10% previously reported.[6–8,27] In the present investigation, it was shown that individuals with HTPR exhibited a 2.78-fold increased susceptibility to secondary ischemic stroke in comparison to those without HTPR. Similarly, patients with T2DM had a 2.64-fold greater risk of secondary ischemic stroke when compared to individuals without T2DM. To the best of our current understanding, this research represents the initial investigation into the association between T2DM, an isolated risk factor for HTPR as determined by the TEG technique, and ISR. HTPR is considered to be a better predictor of adverse clinical outcomes than T2DM. Similarly, after adjusting for covariates in the present study, HTPR had a higher HR for ISR than T2DM. A comprehensive review by Wisniewski et al showed genetic polymorphisms, drug interactions, vascular disease, laboratory data (leukocytes and CRP, etc.), and stroke etiology as influencing factors for clopidogrel resistance.[28] Thus, here we hypothesize that patients' HTPR to clopidogrel is influenced by multiple factors, including genetic polymorphisms, in addition to T2DM, making their platelet adhesion and/or aggregation function stronger and their risk of ischemic event recurrence greater. In future studies, we will enroll more patients in multiple regions and centers to follow up and then do a subgroup analysis of HTPR on ISR. And we will try to use the TEG technique to investigate the effect of risk factors for platelet hyperreactivity on ISR when patients are treated with other antiplatelet agents.

There are several explanations why TEG can reliably identify HTPR caused by the T2DM-mediated decrease of clopidogrel's anti-thrombotic activity and forecast the relationship between HTPR and T2DM with ISR within 90 days of onset. First, patients with diabetes who have persistent hyperglycemia and insulin resistance produce less nitric oxide from their endothelial and platelet cells, which leads to platelet activation and endothelial cell dysfunction.[29] Second, insulin-resistant T2DM patients have high levels of the inflammatory substance IL12,[30] which can activate platelets.[31] Third, endothelial cells damaged by hyperglycemia and insulin resistance release tissue factor,[32] which binds to coagulation factor VII and activates exogenous and endogenous coagulation pathways to form thrombin, which catalyzes an increase in platelet face glycoprotein IIb/IIIa expression (platelet hyperreactivity). PT and APTT in the T2DM cohort were considerably shorter compared to the non-T2DM cohort, as seen in Table 3 of the present investigation. Fourth, insulin receptor substrate-1 is present inside human platelets, and Ferreira et al showed that insulin (possibly through loss of G-protein Gi activity by binding to IRS-1 on platelets) reduces inhibition of cyclic adenosine monophosphate, thereby

inhibiting P2Y12 signaling to reduce platelet responsiveness.[33] Upregulation of the P2Y12 pathway in insulin-resistant T2DM patients leads to increased platelet reactivity, which causes reduced responsiveness to clopidogrel.[33–35] Thus, diabetes is a thrombogenic initiating factor that substantially increases the risk of ischemic recurrence in individuals.[36,37] Briefly, diabetes is characterized by endothelial cell dysfunction and inflammation, which are capable of leading to platelet activation. However, upregulation of the P2Y12 pathway due to insulin resistance can cause impaired anti-platelet action of clopidogrel, which is related to HTPR.

This study has some limitations. First, this study was conducted at a single center and utilized a cohort design with a limited sample size, and even after controlling for confounders, there is still the possibility of bias. Second, this study had some design flaws and did not collect additional clinical baseline characteristics and follow-up information. Third, we did not take into account the role of genetic factors and vitamin D levels in the relationship between HTPR and ISR. Fourth, it is critical to stress that platelet reactivity is a dynamic and complex process. The HTPR in the present investigation, however, was measured at one point in time after admission and is not indicative of long-term platelet reactivity during anti-platelet therapy. In addition, the threshold for HTPR in this study was determined based on the TEG manufacturer's instructions and previous foreign studies, which is not appropriate for the Chinese population.

Conclusion

This study investigated the predictive value of the PIR of TEG on ISR. As the inhibiting impact of clopidogrel on activated platelets is diminished in insulin-resistant T2DM patients, T2DM is an isolated risk factor for HTPR, and both are associated with ISR. Therefore, PIR of TEG can identify individuals at high risk for ISR as early as possible, and this study has the potential to offer more evidence in favor of personalized treatment and secondary preventive efforts.

Data Sharing Statement

The data supporting the findings of this study are available upon reasonable request from the corresponding author.

Ethics Statement

The present study received ethical approval from the Ethics Committee of the First Affiliated Hospital of Anhui Medical University (NO. 2023-13-40) and complied with Helsinki's Declaration. Informed consent was obtained from all patients in this study.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests in this work.

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