Received: 13 April 2020 | Revised: 13 October 2020 | Accepted: 12 November 2020

DOI: 10.1002/hsr2.218

RESEARCH ARTICLE

Health Science Reports Open Access WILEY

Quantitatively monitoring acute ischemic stroke patients post recombinant tissue plasminogen activator treatment

Yonge Liu | Jingting Ma | Qiyang Shi | Shimeng Xin | Haojia Yu | Zilong Liu | Chunsong Pang | Feng Dong | Jinghan Wang

Emergency Laboratory, The second hospital of Dalian Medical University, Dalian, China

Correspondence
Jingting Ma, Emergency Laboratory, the second hospital of Dalian Medical University, No.467 Zhongshan Road, Shahekou District, Dalian 116023, China.
Email: jtingm@outlook.com

Abstract

Background and aims: Thrombolytic therapy is widely used to treat acute ischemic stroke (AIS) patients. As intracerebral hemorrhage is a life-threatening complication of this therapy, monitoring the fibrinolytic and coagulation systems is imperative. However, existing studies on plasmin inhibitor complex (PIC) and thrombin-antithrombin III complex (TAT) mostly apply the enzyme-linked immunosorbent assay (ELISA) method. The aim of this study is to establish the baseline of thrombolytic treatment for AIS patients; to monitor the fibrinolytic and coagulation system following alteplase administration; to ascertain the proper time point to predict intracerebral hemorrhage.

Methods: The method used to assess a patient's intravascular situation, namely chemiluminescence, was used to quantitatively assess the PIC, TAT, and thrombomodulin (TM). Immuno-turbidimetric was used to assess the concentration of D-dimer, fibrin/fibrinogen degradation products (FDP), and the Von Willebrand factor (vWF). The Clauss clotting method was used to assay the activated partial thromboplastin time (APTT), prothrombin time (PT) and FIB.

Results: PIC increased to its peak concentration at 3 hours post intravenous (IV) alteplase infusion and decreased by nearly 50% every 3 hours thereafter. After 24 hours, PIC returned to its normal range, while D-dimer and FDP decreased 3 hours later compared to PIC. PT and APTT exhibited no obvious change during the 24-hour period. TM also exhibited no changes during the treatment.

Conclusion: PIC decreased 3 hours earlier than D-dimer and FDP. The combined test of PIC, D-dimer, and fibrinogen can be used to monitor the fibrinolytic system after the IV alteplase infusion. The use of IV alteplase had no impact on the endothelium. Creating a patient's individual data curve could assist in the prediction of hemorrhagic transformation (HT) and a stroke occurring.

KEYWORDS

acute ischemic stroke, D-dimer, IV alteplase, plasmin-a2 plasmin inhibitor complex, thrombin-antithrombin complex

1 | INTRODUCTION

Intravenous (IV) alteplase is recommended for eligible acute ischemic stroke (AIS) patients.[1] However, the risks of antithrombotic therapy within the first 24 hours after treatment with IV alteplase are uncertain. One of the life-threatening risks is intracerebral hemorrhage.[2-4] Normally, all patients will undertake a noncontrast head Computed Tomography (CT) or brain Magnetic Resonance Imaging (MRI) at 24 hours to rule it out.[5] IV alteplase is a tissue plasminogen activator (t-PA), which magnifies the fibrinolytic system by converting more plasminogen into plasmin.[5] Consequently, monitoring representative markers can provide a clearer view of alterations of a patient's fibrinolytic systems.[6] D-dimer is the fragment of plasmin-cleaved insoluble and cross-linked fibrin, which has been found to be higher in AIS patients on admission 1 week and 1 month after anticoagulant treatment in comparison to healthy subjects.[7] Furthermore, D-dimer has also been proven to be an outcome predictor for ischemic strokes.[8,9] Theoretically, D-dimer is at the downstream of the fibrinolytic system. As such, the activation of D-dimer demonstrates that the fibrinolysis has already occurred. D-dimer is primarily used for the negative exclusion of thrombus.[10-12] Therefore, the sensitivity of the D-dimer reagent required an increase. Thus, the false positive of D-dimer may occur. Apart from fibrin, plasmin also cleaves fibrinogen into several fragments that are referred to as fibrin/fibrinogen degradation products (FDP).[13] Theoretically, assays of FDP could be combined with D-dimer in clinical trials to distinguish this false positive. AIS patients were also found to have an elevated concentration of fibrinogen.[14] In theory, fibrinogen and FDP can also be used to monitor the fibrinolysis. As more plasmin exits in circulation after the usage of IV alteplase, the assays of plasmin concentration can predict the activation of fibrinolysis.[15] However, it is quite difficult to find that studies have illustrated the modification of plasmin in stroke patients. This may be due to the lack of testing methods. An elevated concentration of plasmin-α2 plasmin inhibitor complex (PIC) was found not only within 48 hours following a stroke but also after 1 and 3 weeks.[6] The method used to test PIC was enzyme-linked immunosorbent assay (ELISA). Yet, the accuracy of ELISA is often not ideal. Moreover, this method is time-consuming. Therefore, the feasibility of applying it in clinical trials was relatively low.

Prothrombin time (PT), activated partial thromboplastin time (APTT), and thrombin time (TT) are most widely used in assessing the coagulation protein system in patients.[16] According to guidelines, prior to the application of IV alteplase; PT, and APTT are used to check the eligibility of AIS patients.[1] With regard to the clotting progress, thrombin is the key factor to convert fibrinogen into fibrin and activate platelets.[6] Thrombin-antithrombin III complex (TAT) has been proven to have an association with the outcomes in t-PA treated ischemic stroke patients and to increase the risk of a stroke occurring in atrial fibrillation (AF) patients.[17,18] Similar to PIC, the assays of TAT were also mostly conducted using ELISA.

In terms of the physiological state, the endothelium assumes an integral role in maintaining the anticoagulation system and participating in fibrinolysis.[19,20] On the surface of the endothelial cell, thrombomodulin (TM) binding with protein C can initiate the anticoagulation system with the existence of thrombin.[21] Plasminogen and tissue plasminogen factor bind with endothelial cells for continuous fibrinolysis.[22] Studies have revealed that the concentration of TM was able to represent the degree of damage to the endothelium.[23,24] TM can also inhibit the activation of Factor XIII, which prevented the polymerized fibrin clot from transforming into a covalently cross-linked fibrin clot, a more stable state.[9,25,26] A study also revealed the correlation between serum TM and the risk of atherosclerotic disease.[27] Atherosclerosis is one of the risk factors of AIS.[28] The stroke clot was primarily arterial thrombus.[29] Platelet activation contributes significantly to coagulation.[14] Von Willebrand factor (vWF) helps the adhesion of platelets by connecting to glycoprotein Ib (GPIb) on platelets.[9] The binding between vWF and GPIb has recently become a therapy target.[30] A higher concentration of vWF was found in AIS patients in comparison to healthy people.[31] There was also a study that discovered that plasmin can cleave vWF multimers.[32]

From the latest guidelines, the establishment of data repositories has been newly recommended, therefore, it was necessary to ascertain some laboratory markers, which could be used to improve the quality of ischemic stroke treatment.[1] Stroke is a pathophysiology complex disease.[29] Generally, it is an unbalance of coagulation, fibrinolytic, and anticoagulant systems.[29,33] Thus, in the present study, several markers were selected from three systems in order to evaluate the IV alteplase-post condition of an AIS patient. In order to discover a more accurate result in less time, the chemiluminescence method was used to quantify the concentration of PIC, TAT, and TM for the first time. The researchers attempted to find appropriate markers, which could be used to predict the risk of hemorrhagic transformation (HT) within 24 hours and predict the patient outcomes.

2 | MATERIALS AND METHOD

2.1 | Patients

The present study was performed in the second hospital of Dalian Medical University. From May 2017 to February 2018, 73 patients over 18 years of age participated in this research within 24 hours of symptom onset.

The exclusion criteria: Patients with hemorrhagic stroke, brain parenchymal bleeding, and subarachnoid hemorrhage as confirmed by a CT scan of the brain. Patients with severe heart, kidney, and liver disease. Patients with other severe ongoing diseases.

Based on different medical treatments, patients were divided into two groups. The thrombolysis group included patients within 4.5 hours of symptom onset. In this group, patients were given IV alteplase. The unthrombolysis group contained patients over 4.5 hours of symptom onset and patients within 4.5 hours but who rejected thrombolysis therapy. In this group, IV alteplase was not contained during the treatment.

From the healthy control group, 32 healthy people were selected, including 13 male and 19 females, aged from 23 to 71. This group underwent sampling only once.

The present study was approved by the local ethics committee, approval number: 2017No.102. Verbal informed consent was obtained from patients for their anonymized information to use in this study. All patient data were analyzed anonymously.

2.2 | Clinical assessment

Acute stroke severity was assessed by the national health stroke scale (NIHSS) at emergency inclusion and 24 hours after IV alteplase administration. A follow-up noncontact CT head or MRI brain scan was also conducted on all patients at 24 hours to preclude intracranial hemorrhage (ICH).

The criteria of effective treatment were (a) NIHSS score decreased ≥4 points at 24 hours post-tPA, or (b) NIHSS score of 0 at 24 hours post-tPA.

2.3 | Plasma samples

Overall, 380 samples were tested in the present study. In terms of the thrombolysis group, in order to find appropriate sampling time points, six patients were selected to perform the pretest. During this stage, blood samples were collected at four time points, namely 0 hour (on admission, before IV alteplase injection), 6 hours, 24 hours, and 3 days after the IV alteplase injection. Subsequent to the pretest, blood samples were collected at seven time points, namely 0 hour (on admission, before alteplase injection), 3 hours, 6 hours, 12 hours, 24 hours, 3 days, and 7 days after the IV alteplase injection. In the unthrombolysis group, plasma samples were collected at three time points, namely 0 hour (at inclusion, before any medication treatment), 3 days, and 7 days from admission. Samples were collected into 3-mL vacuum tubes containing 3.2% sodium citrate (0.2 mL/2 mL blood) by immediate centrifugation at 1500*g* for 15 minutes. After the centrifugation procedure, the platelet counts of the plasma samples were 0 to 10×10^9/L. With regard to the samples that could not be tested immediately after centrifugation, the platelet-poor plasma was collected into Eppendorf (EP) tubes and stored at −20°C until test performance (the storage time did not exceed 24 hours, Figure 1).

2.4 | Reagents and method

Measurements of PT, APTT, FIB, TT, D-dimer, FDP, and vWF were performed at Stago STA-R Evolution. The reagents used were STA-Neoplastin Cl Plus⑤, STA-PTT Automate⑤, STA-$CaCl_2$ 0.025 M, STA-Fibrinogen⑤, STA-Thrombin⑤, LIATEST FDP and STA-von

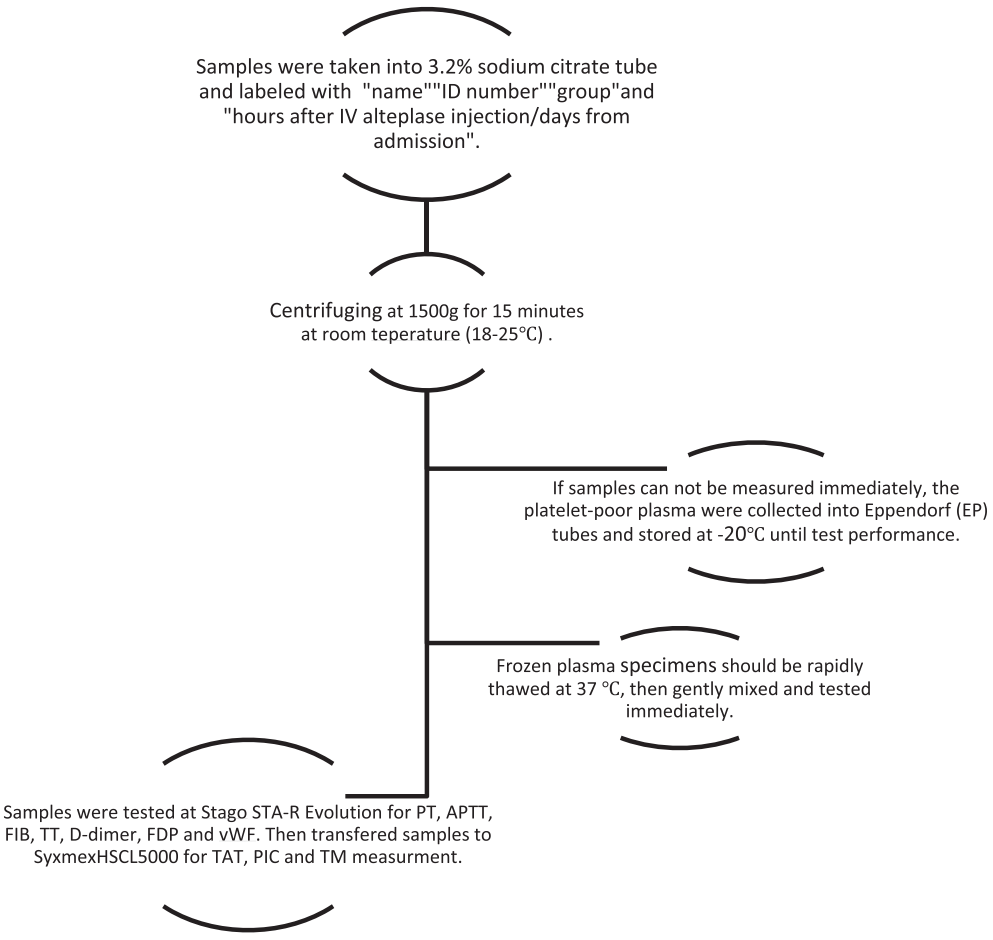

FIGURE 1 The specimen collection, centrifuging, storage and testing processes of samples in two treatment groups

Willebrand factor⑤. The assays of APTT, PT, and TT were conducted using a clotting method. The quantitative determination of FIB concentration in plasma was made by the clotting method of Clauss. The quantitative determination of D-dimer, FDP, and vWF concentration in plasma was determined by the immuno-turbidimetric method.

Measurements of TAT, PIC, and TM were performed at Syxmex HSCL5000. The reagents used were HISCL TAT, HISCL PIC, and HISCL TM from Sysmex. The immuno-turbidimetric method was used to quantitatively test the concentration of TAT, PIC, and TM.

2.5 | Statistics

Graph pad prism7 for Mac was used for statistical calculation. Results are expressed as a mean with SD. The intergroup differences for all markers at seven time points were analyzed by One-way ANOVA and corrected for multiple comparisons (Tukey). This analysis was also used to find group differences among the thrombolysis, unthrombolysis, and healthy control groups. Markers at 0 hour, 3, and 7-day time points of the thrombolysis and unthrombolysis groups were compared using a Mann-Whitney *U* test. In order to identify the sensitivity and the specificity of PIC, D-dimer, and FDP, a receiver operating characteristic (ROC) curve was configured. A *P*-value of <.05 was considered statistically significant.

3 | RESULTS

3.1 | Basic characteristics of study participants

A total of 73 patients (47 male and 26 female patients) with AIS were enrolled in the present study. Patients were divided into two groups based on whether they used IV alteplase during treatment or not. The thrombolysis group was comprised of 38 patients while the unthrombolysis group contained 35 patients. The average age of these patients was 66.39 ± 12.15, ranging from 25 to 89. Among these patients, 58.90% were hypertensive, 47.95% had diabetes mellitus, 32.88% had hyperlipidaemia, 15.07% had a history of AIS, 8.22% had a history of myocardial infarction, 57.53% had atherosclerosis, 20.55% had atrial fibrillation and 6.85% had a history of intracranial hemorrhage. Clinical and demographic features of the study participants are shown in Table 1. In the healthy control group, 32 patients (14 male and 18 female) with an average age of 42.78 ± 12.05, ranged from 22 to 71. The initial NIHSS scores are shown in Table 1. Three patients from thrombolysis group died while one patient from the unthrombolysis group died in the present study.

TABLE 1 Clinical features of patients

	Thrombolysis group	Unthrombolysis group
Gender (male/female)	22/16	25/10
Age (mean ± SD)	68.63 ± 9.96	63.97 ± 13.89
Age < 51	2	6
Age 51-60	5	8
Age 61-70	14	7
Age 71-80	13	11
Age > 80	4	3
Initial NIHSS score		
<10	33	31
10-19	4	4
≥20	1	0
Discharge NIHSS score		
NIHSS score decreased or no change	34	33
NIHSS score increased	0	1
In-hospital mortality	3	1
Combined diseases		
Hypertension	24	19
Hyperlipidaemia	15	9
Atherosclerosis	18	24
Atrial fibrillation	8	7
Diabetes mellitus	18	17
Case history		
History of acute ischemic stroke	5	6
History of myocardial infarction	2	4
History of intracranial hemorrhage	0	5

3.2 | Pretest

As one of the aims for this research is to monitor the fibrinolytic system after the usage of IV alteplase, the time point of sample collecting is the key to the present study. According to existing studies, 24 hours is the clinical assessment point.[1,8] However, the selection of the other time points is various and has no specific criteria. With regard to the pretest period, six patients were selected, collected, and the samples tested at 0 hour (on admission), 6 hours, 24 hours, and 3 days, respectively, to ascertain a tendency of the coagulation and fibrinolytic system after the injection of alteplase. From these six patients, three markers of the fibrinolytic system were discovered, namely PIC, D-dimer, and FDP, which had extremely high levels at 6 hours and decreased gradually after this time point. Moreover, FDP levels returned to the normal range in 24 hours. TAT, PT, and TT all slightly increased at 6 hours and returned to normal from 24 hours. APTT had a similar change as the others but all the values were still below the normal range. It is uncertain whether there were indeed no changes due to the small number of patients. FIB showed a different tendency with others. At 6 hours, FIB decreased by nearly 1 g/L but still remained within the normal range. From 24 hours, FIB rose gradually higher. TM also had the lowest value at 6 hours, and all the values

remained in the normal range. Among these factors, vWF had the highest SD and it was the only factor that had the highest value at 3 days (Table 2).

3.3 | Final results

After the pretest, there were sudden changes from 6 to 24 hours, therefore, 3 hours were added, accumulating to 12 hours for the thrombolysis group. In addition, 7 days were added to the study as the normal treatment period in the selected hospital was 7 days. Changes in all the markers were analyzed in the first 24 hours post IV t-PA treatment and compared between the thrombolysis and unthrombolysis groups at 3 and 7-day time points (Table 3).

4 | COAGULATION SYSTEM

4.1 | Thrombin-antithrombin III complex

When compared with the healthy control group at 0 hour, there was no statistical significance found in either treatment groups. In terms of the thrombolysis group, TAT became significantly higher at 3 hours ($P < .001$, one-way ANOVA) and the SD was 14.92, which is the highest of all time points. At the time point of 6 hours, the value decreased to half at 3 hours. The value decreased slightly at 12 hours and maintained a similar value until 24 hours. However, the value increased slightly at 3 days and decreased at 7 days. The changes from 3 hours to 7 days did not have any statistical significance. The biological reference interval of TAT is <4 ng/mL. After the administration of IV alteplase, the activity of TAT became higher than the normal range until 7 days. At the time point of 7 days, the thrombolysis group appeared to have a lower concentration than the unthrombolysis group. The concentrations of TAT in the unthrombolysis group were similar at 3 and 7 days, which were all greater than 0 hours. There was no difference in the comparisons between the thrombolysis and unthrombolysis groups at 3 and 7 days ($P = .5715$, $P = .1122$, Mann-Whitney U test).

4.2 | Prothrombin time

Comparing the healthy control group at 0 hour, both the thrombolysis and unthrombolysis groups showed statistical significance ($P = .013$ and $P < .0001$, one-way ANOVA). After the injection of IV alteplase, PT did not demonstrate any apparent changes. The normal range of PT was 11 to 14 seconds (locally established). Patients from two-treatment-methods groups both have a slightly longer testing time of PT, and no statistical significance was found between the two groups ($P = .6202$, $P = .378$ at 3 and 7 days, respectively, by Mann-Whitney U test).

4.3 | Activated partial thromboplastin time

APTT from two treatment groups at any time point were all within the normal range (the general reference interval of APTT is 26 to 43 seconds). The comparison among the thrombolysis, unthrombolysis, and healthy control groups demonstrated no significance ($P = .8912$, $P = .3672$, one-way ANOVA). Moreover, there was no statistical significance for group comparison at 3 and 7 days with $P = .8414$ and .6657, respectively (Mann-Whitney U test).

4.4 | Thrombin time

The TT also had no apparent differences between the patients and healthy control group on admission. In the alteplase treatment group, from 3 to 12 hours, TT was longer than that at 0 hour ($P < .0001$, $P < .0001$, and $P = .0005$, one-way ANOVA). During these three time points, TT appeared to have a stable value and longer than the normal

TABLE 2 The results of six thrombolysis patients

	0 h	6 h	24 h	3 days
TAT (ng/mL)	2.8 ± 2.43	4.09 ± 4.12	3.03 ± 3.10	3.97 ± 3.252
PT (S)	13.75 ± 0.63	14.97 ± 0.71	14.25 ± 0.94	14.05 ± 1.14
APTT (S)	39.76 ± 4.97	42.2 ± 5.19	40.97 ± 5.59	39.82 ± 4.36
FIB (g/L)	3.73 ± 0.32	2.89 ± 0.49	3.18 ± 0.50	3.53 ± 0.50
TT (S)	19.83 ± 1.85	22.02 ± 1.99	19.9 ± 1.42	18.87 ± 1.78
PIC (μg/mL)	0.59 ± 0.23	28.27 ± 7.90	1.94 ± 0.96	0.58 ± 0.23
D-dimer (μg/ml)	0.82 ± 0.77	2.96 ± 3.34	1.3 ± 1.36	0.85 ± 0.62
FDP (μg/ml)	2.50 ± 2.28	11.80 ± 17.90	4.45 ± 6.12	2.63 ± 1.23
TM (TU/mL)	9.65 ± 3.45	8.67 ± 2.80	8.97 ± 3.99	10.08 ± 3.11
vWF (%)	128.8 ± 38.31	120 ± 47.77	119.3 ± 52.25	137.2 ± 45.76

Notes: All values are mean ± SD. The general reference interval of these factors in this lab are: TAT (0-4 ng/mL), PT (11-14 s), APTT (26-43S), FIB (2-4 g/L), TT (14-21S), PIC (0–0.8 μg/mL), D-dimer (0-0.5 μg/mL), FDP (0-5 μg/mL), TM (3.8-13.3 TU/mL), vWF (50%-160%).

TABLE 3 The results of the primary test

	0 h	3 h	6 h	12 h	24 h	3 days	7 days	Healthy control
TAT (ng/mL)								2.51 ± 2.32
Thrombolysis group	1.94 ± 1.25	10.77 ± 14.92	5.50 ± 6.34	4.28 ± 3.75	4.26 ± 4.48	5.49 ± 4.70	3.36 ± 2.98	
Unthrombolysis group	2.61 ± 2.78					5.27 ± 4.46	5.24 ± 6.91	
PT (S)								12.52 ± 0.91
Thrombolysis group	14.50 ± 0.95*	15.7 ± 1.48	15.06 ± 3.05	15.47 ± 1.26	15.16 ± 1.01	14.54 ± 0.90	14.86 ± 1.51	
Unthrombolysis group	15.49 ± 4.19***					14.96 ± 1.85	14.74 ± 1.31	
APTT (S)								38.23 ± 3.01
Thrombolysis group	38.86 ± 4.07	41.74 ± 6.85	41.94 ± 5.57	41.21 ± 5.52	40.07 ± 4.43	38.83 ± n;3.96	39.81 ± 3.36	
Unthrombolysis group	40.02 ± 7.57					39.14 ± 5.461	39.48 ± 4.67	
FIB (g/L)								3.011 ± 0.56
Thrombolysis group	3.49 ± 0.95	2.57 ± 0.84	2.60 ± 0.87	2.64 ± 0.84	2.83 ± 0.86	3.37 ± 1.22	3.82 ± 1.13	
Unthrombolysis group	3.56 ± 1.09*					3.827 ± 1.00	4.52 ± 1.62	
TT (S)								18.19 ± 1.43
Thrombolysis group	17.79 ± 0.97	23.60 ± 5.196	23.16 ± 4.77	22.00 ± 4.59	19.59 ± 4.21	18.56 ± 1.48	17.62 ± 1.29	
Unthrombolysis group	20.73 ± 15.67					20.70 ± 14.08	18.3 ± 2.31	
PIC (μg/mL)								0.42 ± 0.16
Thrombolysis group	1.39 ± 2.92	63.70 ± 23.38	37.26 ± 16.56	16.37 ± 7.93	2.815 ± 3.43	0.81 ± 0.45	0.87 ± 0.57	
Unthrombolysis group	0.90 ± 0.47					0.87 ± 0.74	1.00 ± 0.48	
D-dimer (μg/mL)								0.29 ± 0.13
Thrombolysis group	0.59 ± 0.43*	5.97 ± 9.06	5.56 ± 11.44	3.59 ± 6.69	1.91 ± 3.02	1.16 ± 1.09	1.46 ± 2.38	
Unthrombolysis group	0.64 ± 0.60**					0.94 ± 1.10	0.94 ± 0.83	
FDP (μg/mL)								1.34 ± 0.78
Thrombolysis group	2.46 ± 1.29*	28.80 ± 82.81	26.69 ± 62.06	15.16 ± 36.74	6.66 ± 10.94	4.23 ± 3.70	5.12 ± 8.56	
Unthrombolysis group	2.69 ± 2.17**					3.39 ± 3.89	3.31 ± 2.75	
TM (TU/mL)								9.05 ± 1.79
Thrombolysis group	12.61 ± 2.88***	11.18 ± 2.87	14.36 ± 16.06	11.45 ± 2.38	11.24 ± 3.21	11.79 ± 2.62	12.00 ± 2.61	
Unthrombolysis group	11.20 ± 3.55**					11.32 ± 3.30	11.39 ± 3.24	
vWF (%)								102.60 ± 31.28
Thrombolysis group	151.00 ± 79.44*	173.40 ± 101.4	161.90 ± 87.42	142.90 ± 82.58	149.00 ± 83.37	150.60 ± 90.31	161.50 ± 69.63	
Unthrombolysis group	128.50 ± 75.52					124.30 ± 78.90	132.90 ± 75.99	

Notes: All the markers were tested at seven time points. In the first 24 h post IV t-PA treatment, the results are compared between the thrombolysis and unthrombolysis groups at 3 and 7-day time points. All values are shown as mean ± SD. The statistical significance of group comparisons is depicted in this table.

*$P = .05$;

**$P < .01$; ***$P < .005$.

range (the general reference interval of TT is 14 to 21 seconds (locally established]). Twenty-four hours after alteplase administration, TT returned to the normal range. The comparisons between 3 hours with three and 7 days both showed $P < .0001$ (one-way ANOVA). In terms of 6 hours, if compared with 3 and 7 days, the *P*-value was .001 and $P < .0001$, respectively (one-way ANOVA). Statistical significance was also found when comparing 12 hours with 3 and 7 days ($P = .0105$, $P = .0014$, one-way ANOVA). At 3 and 7 days, there was no obvious difference found between the thrombolysis and unthrombolysis groups ($P = .0629$, $P = .2154$, Mann-Whitney *U* test).

5 | FIBRINOLYTIC SYSTEM

5.1 | Plasmin inhibitor complex

With regard to 0 hour, 3, and 7 days, three groups showed no significant differences. In terms of the thrombolysis group, 3 hours after the alteplase injection, PIC increased dramatically ($P < .0001$, one-way ANOVA). In addition, the SD of the 3 hours group was the highest. All other time point concentrations were significantly lower than that at 3 hours and the *P* values were all <.0001 (one-way ANOVA). At 6 hours, the concentration of PIC decreased to nearly half of the concentration at 3 hours. However, the level was still higher than that at the remaining time points (*P* values were all <.0001, one-way ANOVA). Twelve hours after the alteplase infusion, PIC kept reducing dramatically. In a comparison between 24 hours, 3 and 7 days, PIC still maintained a high level ($P = .0003$, $P < .0001$, $P = .0002$, one-way ANOVA). Twenty-four hours after the alteplase injection, PIC remained nearly normal (the general reference interval of PIC is 0-0.8 μg/mL). At the 3 and 7-day time points, the levels of PIC almost returned to normal range and were similar to the unthrombolysis group. In terms of the unthrombolysis group, PIC did not have any changes during the 7 days.

5.2 | Fibrinogen

The patients' level of FIB on admission was higher than the healthy control group while the unthrombolysis group showed statistical significance ($P = .0394$, one-way ANOVA). After the administration of alteplase, FIB reduced slightly at 3 hours when compared with 0 hour ($P = .0062$, one-way ANOVA) and maintained similar results for the subsequent 24 hours (6 hours $P = .0094$, 12 hours $P = .0148$, one-way ANOVA). In the thrombolysis group, the concentration of FIB was lower than that of the unthrombolysis group at 3 and 7 days. The *P*-value was .04 for 3 days while the 7 days *P*-value was .1929 (one-way ANOVA). At 7 days, the unthrombolysis group had a higher level of FIB than at 3 days.

5.3 | D-dimer

The thrombolysis and unthrombolysis groups both had a higher level than the healthy control group, namely $P = .0251$ and $P = .0044$, respectively (one-way ANOVA). In the thrombolysis group, D-dimer concentration increased apparently 3 hours after the alteplase injection ($P = .0278$, one-way ANOVA. The general reference interval is 0-0.5 μg/mL). A similar concentration remained for 6 hours but decreased by nearly 50% at 12 hours and kept decreasing up until 24 hours. However, all these changes were of no statistical significance. After 24 hours, the concentration for D-dimer remained nearly identical. No statistical significance was found between the two treatment groups at 3 and 7 days.

5.4 | Fibrin/fibrinogen degradation products

The thrombolysis and unthrombolysis groups both had a higher level of FDP than the healthy control group, namely $P = .0176$ and $P = .0019$, respectively (one-way ANOVA). Resembling the D-dimer, FDP also increased dramatically at 3 hours after alteplase injection ($P = .0437$, one-way ANOVA) with an extremely high SD. At 6 hours, the concentration had no differences compared to 3 hours. At 12 hours, the concentration of FDP decreased significantly when compared to 6 hours and kept decreasing for the following 12 hours. After 3 days of alteplase treatment, the activity of FDP returned to the normal range (the general reference interval is 0-5 μg/mL). No statistical significance was found between the two treatment groups at 3 and 7 days.

6 | ENDOTHELIUM

6.1 | Thrombomodulin

The thrombolysis and unthrombolysis groups all had a higher concentration of TM than the healthy control group ($P < .0001$ and $P = .0081$, respectively, one-way ANOVA). The usage of alteplase did not influence the activity of TM (*P*-value was all >.5, data not shown here).

6.2 | Von Willebrand factor

The thrombolysis group had a higher level of vWF at 0 hour than the healthy control ($P = .0125$, one-way ANOVA). For each time point of all the groups, vWF has the biggest SD than other markers. Alteplase had no influence on the activity of vWF. The thrombolysis group appeared to have a higher level of vWF than the unthrombolysis group at all time points, but no statistical significance was found (data not shown).

6.3 | Sensitivity and specificity

In the comparison between the accuracy of the D-dimer, FDP, and PIC, at every time point from two treatment groups (see Tables S1

and S2; Figures 2 and 3). In terms of the thrombolysis group, PIC had a higher accuracy than D-dimer and FDP on admission. After the usage of IV alteplase, all these markers had a high sensitivity and specificity. Among them, the area under curve (AUC) of PIC could reach 1.00 at 3, 6, and 12 hours. D-dimer had 100% sensitivity and specificity at 3 hours. The accuracy of FDP appeared slightly lower than the other two markers. At 24 hours, the accuracy of PIC was better than the other two markers. With regard to the unthrombolysis group, PIC appeared to have a slightly higher accuracy at three time points. However, in general, three markers had similar accuracy in two treatment groups at 0 hour, 3, and 7 days. The accuracy of the TAT was also calculated, as shown in Table S3. There were neither obvious differences between the two treatment groups at 0 hours, 3 and 7 days. The sensitivity and specificity demonstrated a literally opposite alternation from 0 hour to 7 days. In terms of sensitivity, it started at approximately 16% and increased to nearly 90% at 7 days. In terms of

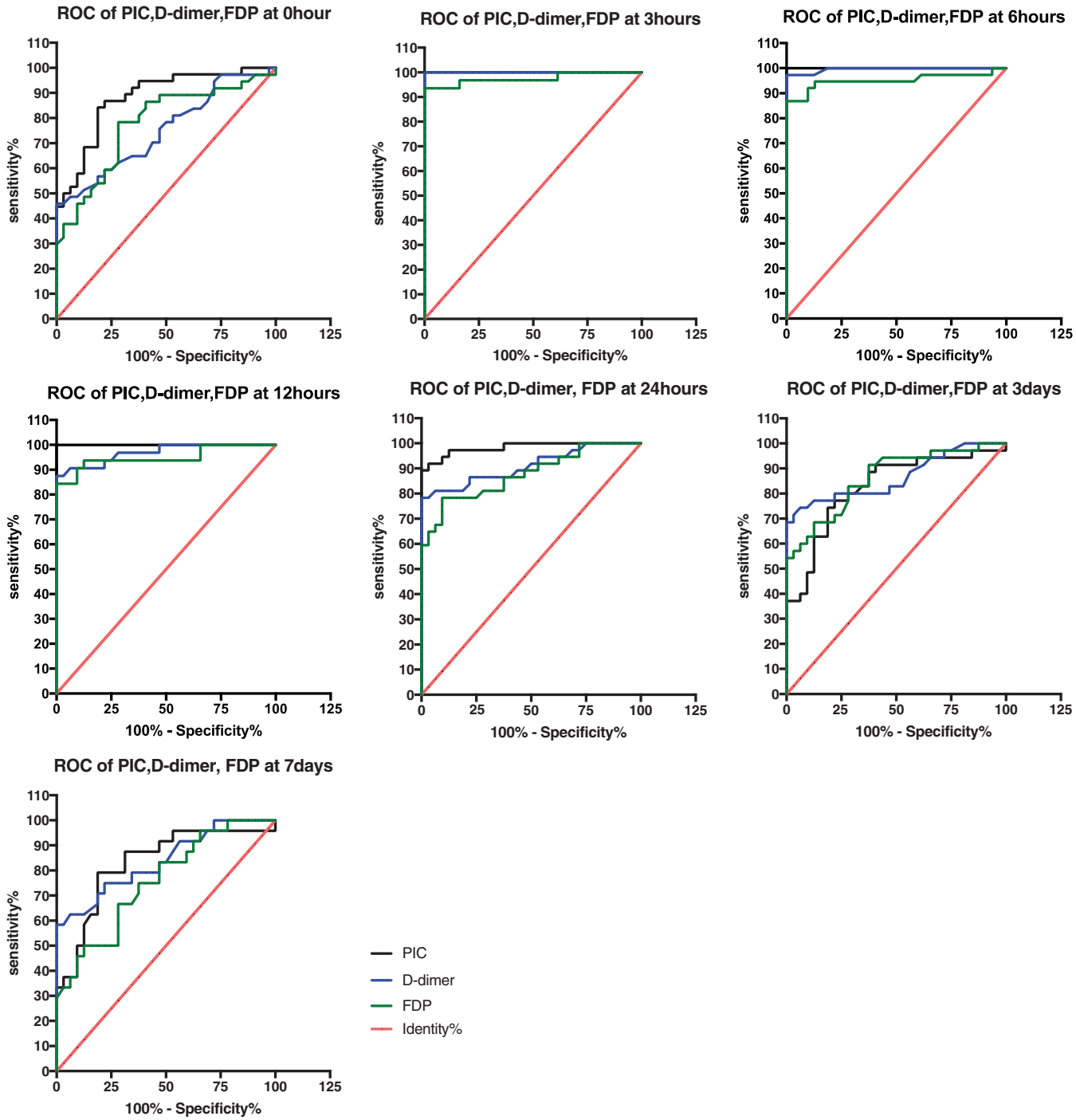

FIGURE 2 The receiver operating characteristic (ROC) curves of thrombolytic group's PIC, D-dimer and FDP at seven time points. The sensitivity and specificity of each time point were illustrated in Table S1

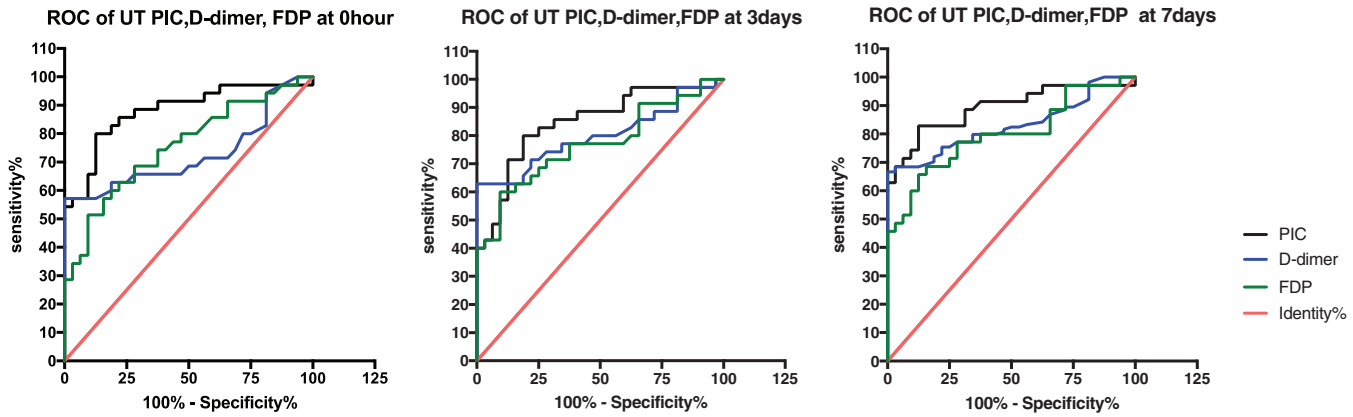

FIGURE 3 The ROC curve for the unthrombolysis treatment group. UT, unthrombolysis treatment group

specificity, it was nearly 100% at 0 hour and decreased to 56.25% at 7 days. In the thrombolysis group, the AUC was over 0.8 at 3 hours and 3 days. In summary, the sensitivity of TAT is not ideal.

7 | DISCUSSION

The usage of alteplase always accompanies the risk of intracerebral hemorrhage. The half-life of t-PA is approximately 3 to 8 minutes following a single infusion, although the biologic half-life is believed to be somewhat longer.[34] This uncertain delay was believed to contribute to hemorrhage after injection. However, due to methodological limitations, it has been commonly accepted that thrombolysis treatment is lack of quantitative outcome measurements. Numerous existing studies attempted to diagnose AIS and predict the HT by different markers, but the results of those studies were not ideal.[35-39]

In this study, we used immuno-turbidimetric method to quantitatively test PIC, TAT, and TM. As theoretically, plasmin could be increased by the usage of t-PA, the primary result of the present study was that all the patients had a peak level of PIC at 3 hours after t-PA infusion, however, the peak concentrations varied. This diversity may be due to different individual physiological affection of IV alteplase. With regard to patients with positive outcomes, even if their PIC reached extremely high concentrations, it would decrease sharply during the subsequent few hours and the pattern of decreasing was similar. In summary, PIC reduced approximately 50% every 3 hours from 3 to 12 hours. The diversities decreased as well. After 24 hours, PIC basically maintained in the individual's normal state. Therefore, the usage of IV alteplase only affected plasminogen activation within the first 24 hours. Moreover, the IV alteplase-treated group had similar PIC concentrations to the non IV alteplase-treated group at 3 and 7 days. Thus, if patients had different decreased patterns, the outcome may have been different and HT may have occurred. In the present study, one patient had an even higher level of PIC at six rather than 3 hours; the level of PIC of four patients decreased slightly or remained identical from 3 to 6 hours (decreased less 20%). Among them, two died during hospitalization.

D-dimer is a stable marker after ischemic stroke. It is more stable than TAT, prothrombin fragments 1 + 2 (F1 + 2).[37,38] It is worth considering if any differences could be found in a comparison between PIC and D-dimer. In a comparison between two AIS groups (on admission) with the healthy control group, in contrast to the slight increase in PIC, the AIS patients' D-dimer were significantly higher than the healthy group. One study illustrated that a higher level of D-dimer indicates a high severity and larger infarct volume.[38] Perhaps due to the limited number of patients and few severe stroke patients, this correlation was not apparent in the present study. After 3 hours of t-PA injection, D-dimer increased nearly six times and remained at a peak level till 6 hours. In contrast to PIC, D-dimer barely decreased from 3 to 6 hours. The high concentration of plasmin concentration in circulation may account for this.[13] As the half-life of plasmin is only 0.1 seconds, t-PA is as long as 4 minutes. Furthermore, the half-life of plasminogen was 2.2 days.[19] Thus, post IV alteplase injections, t-PA was under high concentration, it could continue transforming plasminogen into plasmin.[13] Although PIC reduced to 50% of peak concentration at 6 hours, the concentration was still at a high level. D-dimer is the final fragment of fibrin cleaved by plasmin.[7,10] According to the data, there was a positive correlation between PIC and D-dimer. Therefore, a similar D-dimer concentration was obtained at 3 and 6 hours. As such, the reduction occurred 3 hours later in D-dimer than PIC. The speed was similar to PIC, nearly 50% at every time point from 6 hours to 3 days. As the samples were not tested between 3 and 6 hours, resembling the delayed reduction, the actual peak may also appear later than 3 hours. Further research is required to confirm this notion. Like PIC, the high concentration also combined with the high individual diversity in D-dimer. The concentration and SD reduced from 6 hours to 24 hours. However, from 24 hours to 7 days, the D-dimer remained at approximately 1 μg/mL. From the data, at the end of the treatment period after therapy, PIC could have a lower concentration than the initial one. One reason for this situation may still be the influence of high-level plasmin. Another reason could be that the cross-reactivity with FDP could contribute to percentages of false positive.[40]

FDP has been reported as elevated in ischemic stroke patients and reduced in the convalescent phase.[41] In the present study, an elevated FDP level was also found in AIS patients. Like D-dimer, FDP was also higher in AIS patients than the healthy group on admission. In terms of patients who had several combined comorbidities, old age, or had surgery within a year, it was recommended to select one of them to confirm the patient's fibrinolytic system condition before the IV alteplase injection. Following the usage of IV alteplase, FDP increased almost 30 times with an extremely high SD (82.81) at 3 hours. The alternation of FDP in the present study was essentially similar to D-dimer and a delayed reduction occurred as well. Combined data from PIC and D-dimer, patients' fibrinolytic system indicated a variety of affections for t-PA, however, the patterns were similar. Twenty-four hours was the endpoint of the fibrinolytic system activated extremely. A positive correlation was found in each two of PIC, D-dimer, and FDP. In summary, within the first 24 hours, FDP is not necessarily tested. Thus, we conclude PIC and D-dimer were able to represent an activated fibrinolytic system. As discovered, every reduction between two time points had statistical significance and PIC basically represented kinetics of IV alteplase in patients' circulation. With regard to the late stage of hospitalization, we think FDP could be used to distinguish D-dimer false positives. The methods used to test PIC, D-dimer, and FDP were different. Thus, the accuracy of each aspect was tested. Each had a better performance when the fibrinolytic system was activated than in the inactivated stage. Within the first 24 hours after IV alteplase infusion, they all had high accuracy. It was also discovered that PIC had the best sensitivity among them. The accuracy of D-dimer and FDP had no obvious differences, while D-dimer had slightly higher accuracy. With regard to PIC and D-dimer, the sensitives and specificities were compared between the thrombolysis treatment group and the unthrombolysis group at 3 and 7 days and no higher accuracy was found in the thrombolysis group. As the effect of IV alteplase on the concentration only continues for 24 hours, these markers could be more accurate when the fibrinolytic system is activated. Considering all these aspects, testing PIC and D-dimer during the first 24 hours after IV alteplase infusion was considered in order to monitor the fibrinolytic system by representing upstream and downstream.

The purpose of using t-PA was to dissolve clots instead of coagulation factors. Plasmin does not only dissolve fibrin but also fibrinogen.[13] From the data, fibrinogen had decreased slightly after the t-PA injection, and there were insignificant diversities among all the patients. Among all the markers tested, fibrinogen had the lowest SD. As stated, when t-PA is infused into circulation, it would bind with fibrin and focus on the area of the clot.[42] Hence, after fibrin dissolves, the remaining t-PA can continue reacting with fibrinogen. The amount of IV alteplase used in this hospital was 0.9 mg/kg according to the guideline.[1] From the data, the affection of t-PA in patients was different. Thus, while even using a safe amount, some patients could also have HT. On this basis, it is assumed that if a patient exhibited an extremely low fibrinogen concentration, this patient may have a high risk of HT. One patient who died of HT had a fibrinogen level of 0.6 g/L at 6 hours, which was much lower than other positive outcome patients. With regard to this patient who had no improvement after IV alteplase infusion, the intraarterial mechanical thrombectomy was undertaken. Considering that the patient was an 80-year-old female, the combination of t-PA and heparin used during thrombectomy contributed to the irreversible imbalance of fibrinolysis and coagulation systems. After thrombectomy, this female patient kept bleeding until she died. A large transfusion could not reverse the situation. Therefore, it is suggested that testing fibrinogen together with PIC and D-dimer could be more comprehensive.

According to the guideline, before the infusion of IV alteplase, PT, and APTT should be tested.[1] Only those with normal PT and APTT were eligible to have IV alteplase. In this research, normal coagulation tests were conducted, which contain PT, APTT, and TT at every testing time point. From the results, positive outcome patients had slightly longer PT and APTT, but it was still under the normal range within the 24 hours. Fibrinogen slightly decreased and as a result, TT was a little longer than the normal range. Basically, the normal coagulation tests were not efficiently used in monitoring IV alteplase treatment. In terms of the deceased patient, PT, APTT, and TT increased when HT occurred. However, after the transfusion, each reduced to nearly normal range although the patient still had severe bleeding. TAT was also tested simultaneously but the level was extremely high. The reason for this was the clotting time of PT, TT, and APTT were based on the concentration and activity of coagulation factors and fibrinogen. A large transfusion can lead to a fake normal clotting time. However, the measurement of TAT was based on antigen-antibody reaction. Thus, TAT, as a marker of the coagulation system can be used to check patients' real coagulation system situation after transfusion.

In the present study, two markers were selected to depict the endothelium. AIS patients exhibited significantly higher TM than the healthy control group. The thrombolysis group had a higher vWF than healthy patients. TM essentially had no changes during the 24 hours. Comparing the thrombolysis group with the unthrombolysis group, no differences were identified. As TM was able to represent the endothelium damage,[23] it was assumed that the usage of IV alteplase had no effect on the endothelium, although there was one study that had found a high level of vWF in AIS.[31] In this research, the concentrations of vWF in patients varied. In contrast to other markers, a clear trend of its variance could not be identified. This fluctuation may be associated with the patients' diverse reaction to IV alteplase. Notwithstanding, there was not any clear relationship between vWF and treatment outcome in this research. Thus, in terms of future research, TM in AIS patients who had a high NHISS score should be analyzed.

The limitations of the present study are the limited number of patients and their criteria. As the thrombolytic group needs multiple sample collections, which needs a good cooperation of doctors, nurses, technicians, and the patients. However, it is quite difficult to accomplish all the collection timepoint for each patient. The whole scale was nearly 120, but only 79 patients complete every timepoints in the present study. For next stage study, expanding the sample scale is necessary. Besides, most patients included in this research had a low NHISS score, which indicated that theoretically, they had a lower

probability of having a poor outcome. The results of the present study could provide an indication for future AIS studies. The application of the quality test in testing PIC, TAT, and TM can provide an opportunity to improve the management of AIS patients.

8 | CONCLUSION

The combined test of PIC, D-dimer, and fibrinogen could be used to quantitatively reflecting the impaction of IV alteplase on fibrinolytic system. For well-outcome patients, the usage of IV alteplase did not damage endothelium condition.

ACKNOWLEDGMENTS

We would like to thank all parents who cooperated in the research. This work was supported by the Medical Sciences Research program of Dalian of China, Grant/Award Number: 1812032.

FUNDING

The author(s) received financial support from the second hospital of Dalian Medical University for the sample collection, cost of reagents, material, and publication of this article.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHOR CONTRIBUTIONS

Conceptualization: Jingting Ma, Yonge Liu, Xinshi Meng, Haojia Yu
Data Curation: Jingting Ma, Qiyang Shi
Formal Analysis: Jingting Ma
Funding Acquisition: Yonge Liu
Investigation: Jingting Ma, Qiyang Shi, Haojia Yu, Zilong Liu, Pangchun Song, Dong Feng, Dan Wu, Jinghan Wang
Methodology: Jingting Ma, Yonge Liu, Qiyang Shi
Project Administration: Yonge Liu, Xinshi Meng
Resources: Yonge Liu, Xinshi Meng
Supervision: Yonge Liu, Xinshi Meng
Validation: Jingting Ma, Qiyang Shi, Wenting Lao
Visualization: Jingting Ma
Writing—Original Draft: Jingting Ma
Writing—Review & Editing: Jingting Ma

All authors have read and approved the final version of the manuscript.

Jingting Ma had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

TRANSPARENCY STATEMENT

The lead author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article supplementary materials.

ORCID

Jingting Ma https://orcid.org/0000-0001-5426-5790

REFERENCES

1. Furie KL, Jayaraman MV. 2018 Guidelines for the early management of patients with acute ischemic stroke. *Stroke*. 2018;49:509-510. https://doi.org/10.1161/STROKEAHA.118.020176.
2. Paciaroni M, Agnelli G, Corea F, et al. Early hemorrhagic transformation of brain infarction: rate, predictive factors, and influence on clinical outcome: results of a prospective multicenter study. *Stroke*. 2008; 39(8):2249-2256.
3. Moriya Y, Takahashi W, Kijima C, et al. Predictors for hemorrhagic transformation with intravenous tissue plasminogen activator in acute ischemic stroke. *Tokai J Exp Clin Med*. 2013;38(1):24-27.
4. Fagan SC, Lapchak PA, Liebeskind DS, Ishrat T, Ergul A. Recommendations for preclinical research in hemorrhagic transformation. *Transl Stroke Res*. 2013;4(3):322-327.
5. Draxler DF, Medcalf RL. The fibrinolytic system-more than fibrinolysis. *Transfus Med Rev*. 2015;29(2):102-109.
6. Kataoka S, Hirose G, Hori A, Shirakawa T, Saigan T. Activation of thrombosis and fibrinolysis following brain infarction. *J Neurol Sci*. 2000;181(1):82-88.
7. Haapaniemi E, Soinne L, Syrjälä M, Kaste M, Tatlisumak T. Serial changes in fibrinolysis and coagulation activation markers in acute and convalescent phase of ischemic stroke. *Acta Neurol Scand*. 2010; 110(4):242-247.
8. Ungerstedt JS, Grenander A, Bredbacka S, Blombäck M. Clotting onset time may be a predictor of outcome in human brain injury: a pilot study. *J Neurosurg Anesthesiol*. 2003;15(1):13-18.
9. Renshaw A. Henry's clinical diagnosis and management by laboratory methods. *Adv Anat Pathol*. 2007;14(2):147.
10. Johnson ED, Schell JC, Rodgers GM. The D-dimer assay. *Am J Hematol*. 2019;94:833-839.
11. Weitz JI, Fredenburgh JC, Eikelboom JW. A test in context: D-dimer. *J Am Coll Cardiol*. 2017;70(19):2411-2420.
12. Halaby R, Popma CJ, Cohen A, et al. D-dimer elevation and adverse outcomes. *J Thromb Thrombolysis*. 2015;39:55-59.
13. Marder VJ, Budzynski AZ. Degradation product of fibrinogen and crosslinked fibrin-projected clinical applications. *Thromb Diath Haemorrh*. 1974;32(2):49-56.
14. Rooth E, Wallen NH, Blombäck M, He S. Decreased fibrin network permeability and impaired fibrinolysis in the acute and convalescent phase of ischemic stroke. *Thromb Res*. 2011;127(1):51-56.
15. Cardenas JC, Matijevic N, Baer LA, Holcomb JB, Cotton BA, Wade CE. Elevated tissue plasminogen activator and reduced plasminogen activator inhibitor promote hyperfibrinolysis in trauma patients. *Shock*. 2014;41(6):514-521.
16. Gosselin RC, Adcock D, Dorgalaleh A, et al. International Council for Standardization in Haematology recommendations for hemostasis critical values, tests, and reporting. *Semin Thromb Hemost*. 2020;46:398-409.
17. Israel FC, Maite M, Josep M, et al. Lower concentrations of thrombin-antithrombin complex (TAT) correlate to higher recanalisation rates among ischaemic stroke patients treated with t-PA. *Thromb Haemost*. 2009;102:759-764.

18. Wu N, Chen X, Cai T, et al. Association of inflammatory and hemostatic markers with stroke and thromboembolic events in atrial fibrillation: a systematic review and meta-analysis. *Can J Cardiol*. 2015;31(3):278-286.
19. McKenzie SB. *Clinical Laboratory Hematology: Pearson New International Edition*. New Jersey, NJ: Pearson Education, Inc; 2013.
20. Lichtman MA, Kaushansky K, Prchal JT, Levi MM, Burns LJ, Armitage JO. *Williams Manual of Hematology*. New York, NY: McGraw Hill Professional; 2017.
21. Sadler JE. Thrombomodulin structure and function. *Thromb Haemost*. 1997;78(1):392-395.
22. Barnathan ES, Kuo A, Keyl HVD, McCrae KR, Larsen GR, Cines DB. Tissue-type plasminogen activator binding to human endothelial cells. Evidence for two distinct binding sites. *J Biol Chem*. 1988;263(16):7792-7799.
23. Seigneur M, Dufourcq P, Conri C, et al. Plasma thrombomodulin: new approach of endothelium damage. *Int Angiol*. 1993;12(4):355.
24. Esmon CT. The interactions between inflammation and coagulation. *Br J Haematol*. 2010;131(4):417-430.
25. Polgár J, Léránt I, Muszbek L, Machovich R. Thrombomodulin inhibits the activation of factor xiii by thrombin. *Thromb Res*. 1986;43(5):585-590.
26. Koncz Z, Bagoly Z, Haramura G, Mezei ZA, Muszbek L. Thrombomodulin-dependent effect of factor V Leiden mutation on the cross-linking of α2-plasmin inhibitor to fibrin and its consequences on fibrinolysis. *Thromb Res*. 2012;130(3):528-534.
27. Dohi Y, Ohashi M, Sugiyama M, Takase H, Sato K, Ueda R. Circulating thrombomodulin levels are related to latent progression of atherosclerosis in hypertensive patients. *Hypertens Res*. 2003;26(6):479-483.
28. Chamorro A. Role of inflammation in stroke and atherothrombosis. *Cerebrovasc Dis*. 2004;17(Suppl. 3):1-5.
29. Grysiewicz RA, Thomas K, Pandey DK. Epidemiology of ischemic and hemorrhagic stroke: incidence, prevalence, mortality, and risk factors. *Neurol Clin*. 2008;26(4):871-895.
30. Frederik D, De MSF. The VWF-GPIb axis in ischaemic stroke: lessons from animal models. *Thromb Haemost*. 2016;116(10):597-604.
31. Muuronen AT, Taina M, Onatsu J, et al. VWF correlates with visceral and pericardial adipose tissue in patients with a recent stroke of suspected cardiogenic etiology. *PLoS ONE*. 2017;12(6):e0178508.
32. van der Vorm LN, Remijn JA, de Laat B, Huskens D. Effects of plasmin on von Willebrand factor and platelets: a narrative review. *TH Open*. 2018;2(2):e218-e228.
33. Ingall T. Stroke—incidence, mortality, morbidity and risk. *J Insur Med*. 2004;36(2):143-152.
34. Ranby M, Bergsdorf N, Norrman B, Suenson E, Wallén P. Tissue plasminogen activator kinetics. In: Davison JF, Bachmann F, Bouvier CA, EKO K, eds. *Progress in Fibrinolysis*. Vol 6. New York, NY: Churchill-Livingstone; 1982:182.
35. An SA, Kim J, Kim OJ, et al. Limited clinical value of multiple blood markers in the diagnosis of ischemic stroke. *Clin Biochem*. 2013;46(9):710-715.
36. Ng GJL, Quek AML, Cheung C, Arumugam TV, Seet RCS. Stroke biomarkers in clinical practice: a critical appraisal. *Neurochem Int*. 2017;107:11-22.
37. Barber M, Langhorne P, Rumley A, Lowe GDO, Stott DJ. D-dimer predicts early clinical progression in ischemic stroke: confirmation using routine clinical assays. *Stroke*. 2006;37(4):1113-1115.
38. Zi WJ, Shuai J. Plasma D-dimer levels are associated with stroke subtypes and infarction volume in patients with acute ischemic stroke. *PLoS One*. 2014;9:e86465.
39. Simats A, García-Berrocoso T, Montaner J. Neuroinflammatory biomarkers: from stroke diagnosis and prognosis to therapy. *Biochim Biophys Acta*. 2016;1862(3):411-424.
40. CLSI. *Quantitative D-Dimer for the Exclusion of Venous Thromboembolic Disease; Approved Guideline. CLSI Document H59-A*. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.
41. Rothwell PM, Howard SC, Power DA, et al. Fibrinogen concentration and risk of ischemic stroke and acute coronary events in 5113 patients with transient ischemic attack and minor ischemic stroke. *Stroke*. 2004;35(10):2300-2305.
42. Thelwel C, Longstaf C. The regulation by fibrinogen and fibrin of tissue plasminogen activator kinetics and inhibition by plasminogen activator inhibitor 1. *J Thromb Haemost*. 2007;5(4):804-811.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Liu Y, Ma J, Shi Q, et al. Quantitatively monitoring acute ischemic stroke patients post recombinant tissue plasminogen activator treatment. *Health Sci Rep*. 2020;4:e218. https://doi.org/10.1002/hsr2.218