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·Original Article·

Increased globulin and its association with hemorrhagic transformation in patients receiving intra-arterial thrombolysis therapy

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ABSTRACT

Previous studies have identified a diverse set of predisposing factors for the occurrence of hemorrhagic transformation (HT), but the independent clinical predictors of HT after intra-arterial thrombolysis have not been determined. In this retrospective study, we investigated the characteristics of patients with or without HT who had received intra-arterial thrombolysis therapy, using biochemical analysis, renal function test, routine blood test, blood lipid test, coagulation blood test, liver function test, random blood glucose test, time-window for intra-arterial thrombolysis, recanalization, National Institutes of Health Stroke Scale (NIHSS) score and systolic blood pressure before intra-arterial thrombolysis. The mortality rates were similar in the HT and non-HT groups (P = 0.944). In the single-factor analysis, patients with a higher globulin level (P <0.002), prothrombin time activity percentage (PTA; P = 0.026), and NIHSS score (P = 0.002), had a significantly increased risk of developing HT. In the multifactor logistic regression model involving globulin level, PTA, white blood cell count, and NIHSS score, the globulin level (P <0.001; OR, 1.185; 95% confidence interval [CI], 1.090–1.288), PTA (P = 0.018; OR, 1.016; 95% CI, 1.003–1.029), white blood cell count (P = 0.025; OR, 1.097; 95% CI, 1.012–1.190) and NIHSS score (P = 0.003; OR, 1.097; 95% CI, 1.031–1.166) were significantly increased in the HT group. The increase in globulin level is an independent risk factor for HT in patients receiving intra-arterial thrombolysis. The possible mechanisms may involve inflammatory cytokines, matrix metalloproteinase 9, and positive acute-phase reactants synthesized by the liver.

Keywords: hemorrhagic transformation; intra-arterial thrombolysis; globulin

INTRODUCTION

Hemorrhagic transformation (HT) is a common complication of intra-arterial thrombolysis for the treatment of acute ischemic stroke, with an estimated incidence rate of 18–71%[1, 2], depending on factors such as the thrombolytic agent used and the route of administration[1, 3, 4]. Recent studies showed that different subtypes of HT may have a different prognosis, some having a higher recanalization rate and better outcome[5],while some are associated with very poor clinical outcomes[6]. So far, a diverse set of predisposing factors for the occurrence of HT has been identified, including a decreased level of total cholesterol[7], poor collateral circulation[2], a high National Institutes of Health Stroke Scale (NIHSS) score, a low platelet count, and a high glucose level[3], but the independent clinical predictors of HT after intra-arterial thrombolysis have not yet reached consensus. Identification of pretreatment predictors of HT may help in selecting appropriate patients, which would limit complications and clarify the possible

mechanisms of HT. This study aimed to investigate the independent clinical predictors of HT in patients receiving intra-arterial thrombolysis therapy and to raise awareness of the selection of suitable patients.

METHODS

Participants

We performed a retrospective study in patients receiving intra-arterial thrombolysis. Intra-arterial thrombolysis was considered when a patient presented with acute cerebral ischemia and was believed by a neurologist to potentially benefit from the therapy. Antiplatelet and anticoagulant therapies are not the contraindications of intra-arterial thrombolysis, but receiving anticoagulant therapy with a simultaneous international normalized ratio (INR) >1.5 is a contraindication. The time-window of intra-arterial thrombolysis for anterior circulation occlusions is within 6 h of symptom onset, and for posterior circulation occlusions is 24 h. Patients were included in this study if they (1) presented with symptoms of acute cerebral ischemia, (2) received intra-arterial thrombolysis therapy, (3) had a computed tomography (CT) scan on admission that ruled out the possibility of having a cerebral hemorrhage and had a repeated CT scan within 24 h to evaluate the presence of HT, and (4) underwent thorough physical and laboratory tests before receiving intra-arterial thrombolysis therapy. Patients receiving combined intravenous and intra-arterial treatments were excluded. From March 1, 2004 to November 30, 2011, 216 patients receiving intra-arterial thrombolysis were recruited from the First Norman Bethune Hospital of Jilin University. The study design was approved by the Ethics Committee of the First Norman Bethune Hospital of Jilin University.

Thrombolytic Procedure

Cerebral angiography was performed using a transfemoral approach. A diagnostic angiogram was used to determine the target vessels, and the thrombolytic agent (urokinase up to a total dose of 1 250 000 IU, or recombinant tissue plasminogen activator (rt-PA) up to a total dose of 20 mg) was delivered through a microcatheter. A small dose of thrombolytic agent was delivered distal to the thrombus. Then the catheter was retracted into the proximal thrombus, where a small dose of thrombolytic agent was injected. After that, a large dose was infused proximal to the occlusion. Mechanical thrombus disruption was performed during the operation according to the situation. Diagnostic angiograms were performed at regular intervals to assess recanalization. The thrombolytic agent infusion was discontinued when the following occurred: (1) adequate revascularization was achieved; (2) partial or no recanalization occurred but the maximal safe dose of thrombolytic agent had been administered; (3) bleeding out of contrast material was noted; and (4) there was suspicion that HT may have occurred[3].

Definition of HT

All CT scans were evaluated by a neuroradiologist blinded to the clinical characteristics of the stroke and its progress toward HT. HT was defined as displaying any degree of hyperdensity within the low attenuation area[7], including (1) hemorrhagic infarction 1 (HI1): small petechiae along the margins of the infarct; (2) hemorrhagic infarction 2 (HI2): confluent petechiae within the infarcted area but without a space-occupying effect; (3) parenchymal hemorrhage 1 (PH1): hematoma ≤30% of the infarcted area with some slight space-occupying effect; and (4) parenchymal hemorrhage 2 (PH2): hematoma >30% of the infarcted area with a substantial space-occupying effect according to the European Cooperative Acute Stroke Study classification[8]. The patients were divided into HT and non-HT groups. Symptomatic HT was defined as any type of intracranial hemorrhage on any post-treatment imaging after the start of thrombolysis and NIHSS deterioration of ≥4 from baseline, or from the lowest value within 7 days, or leading to death[9].

Laboratory Tests and Other Predictors

Biochemical analysis (calcium, sodium, potassium, and chloride levels), renal function test (urea nitrogen and creatinine levels), routine blood test (white blood cell count and platelet count), blood lipid test (total cholesterol, triglyceride, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol levels), coagulation blood test (activated partial thromboplastin time, INR, and prothrombin time activity percentage [PTA]), liver function test (aspartic aminotransferase, alanine aminotransferase, alkaline phosphatase, r-glutamyl transpeptidase, cholinesterase, total protein, albumin, and globulin levels),

and random blood glucose test were conducted. The other predictors were the time-window for intra-arterial thrombolysis, recanalization, NIHSS score, and systolic blood pressure before intra-arterial thrombolysis.

Statistical Analysis

All data were analyzed with the Statistical Program for Social Sciences version 17.0 (SPSS; IBM, West Grove, PA). The measurement data are expressed as the mean ± SD or median (interquartile range) and analyzed with Student's *t*-test and the Mann-Whitney U test to identify univariate predictors of HT. The count data are expressed as the rate (percentage) and analyzed with the χ^2 and Fisher exact tests. A multifactorial logistic regression model was performed to identify independent predictors of HT.

RESULTS

Study Population and Epidemiologic Data

In the 216 patients recruited, the incidence rate of HT was 19.0% (41/216). Among the 41 patients with HT, 15 (36.6%) were with HI1, 17 (41.5%) with HI2, 6 (14.6%) with PH1, and 3 (7.3%) with PH2; 9 (22.0%) were symptomatic HT and 32 (78.0%) were asymptomatic HT; mortality rate was 19.5% (8/41). The incidence rate of non-HT was 81.0% (175 of 216), and the mortality rate among them was 20% (35 of 175). Other population data are shown in Table 1.

Risk Factors in HT and Non-HT Groups

We did not find any significant difference in age, sex, history of stroke, hypertension, diabetes mellitus, hyperlipidemia, coronary artery disease and atrial fibrillation, smoking, alcohol consumption, antiplatelet and anticoagulant therapies before receiving intra-arterial thrombolysis therapy, thrombolytic agent, time-window for intra-arterial thrombolysis, recanalization, or systolic blood pressure between the HT and non-HT groups. However, the NIHSS score was significantly higher in the HT group than in the non-HT group (z = -3.075, P = 0.002) (Table 1). In addition, the mortality rates in the two groups were similar (t = 0.005, P = 0.944).

The laboratory measurements prior to intra-arterial

Table 1. Comparisons of risk factors in HT and non-HT groups

Factors	HT group (n = 41)	Non-HT group (n = 175)	$\chi^2(t, z)$	P
Male	27 (65.9%)	127 (72.6%)	0.733	0.392
Age (year)	60.05 ± 10.91	58.21 ± 10.62	−0.995	0.321
History of stroke	9 (22.0%)	48 (27.4%)	0.513	0.474
Hypertension	28 (68.3%)	121 (69.1%)	0.011	0.916
Diabetes	9 (22.0%)	40 (22.9%)	0.016	0.901
Hyperlipidemia	10 (24.4%)	33 (18.9%)	0.638	0.425
Coronary artery disease and atrial fibrillation	16 (39.0%)	51 (29.1%)	1.516	0.218
Smoking	10 (24.4%)	39 (22.3%)	0.084	0.772
Alcohol consumption	5 (12.2%)	30 (17.1%)	0.599	0.439
Antiplatelet therapy	19 (46.3%)	62 (35.4%)	1.688	0.194
Anticoagulant therapy*	3 (7.3%)	5 (2.9%)		0.178
rt-PA	7 (17.1%)	18 (10.3%)	0.906	0.341
Urokinase	34 (82.9%)	157 (89.7%)	0.906	0.341
Time window (min)	300 (270–405)	300 (240–382)	−0.525	0.599
Recanalization	33 (80.5%)	144 (82.3%)	0.073	0.788
NIHSS score	12 (11–17)	11 (9–13)	−3.075	**0.002**
Systolic blood pressure (mmHg)	149.53 ± 24.43	155.59 ± 21.61	1.701	0.090

*The Fisher exact test was used to quantify the differences between HT and non-HT groups. rt-PA, recombinant tissue plasminogen activator.

thrombolysis were included in the single-factor analysis. Patients with a higher globulin or PTA level had a significantly increased risk of developing HT (Table 2). For the white blood cell count, the difference between groups showed a trend close to significance (P = 0.067) (Table 2). In the multifactorial logistic regression model, we included

Table 2. Single-factor analysis of laboratory data

	HT group (n=41)	Non-HT group (n=175)	t (z)	P
Calcium (mmol/L)	2.32 ± 0.22	2.31 ± 0.43	−0.067	0.947
Sodium (mmol/L)	138.32 ± 3.97	138.42 ± 3.79	0.158	0.874
Potassium (mmol/L)	3.89 ± 0.39	3.89 ± 0.41	−0.047	0.963
Chloride (mmol/L)	103.66 ± 3.55	102.80 ± 4.26	−1.198	0.232
Urea nitrogen (mmol/L)	5.65 (4.40–6.56)	5.00 (4.02–6.50)	−1.36	0.174
Creatinine (μmol/L)	84.10 ± 23.19	80.44 ± 36.28	−0.617	0.538
White blood cell count (10^9/L)	12.21 ± 5.39	10.81 ± 4.12	−1.841	0.067
Platelet count (10^9/L)	222.49 ± 49.10	211.83 ± 61.87	−1.030	0.304
Total cholesterol (mmol/L)	5.41 ± 1.40	5.07 ± 1.24	−1.599	0.111
Triglyceride (mmol/L)	1.54 (0.88–2.49)	1.27 (0.81–1.88)	−1.265	0.206
High density lipoprotein (mmol/L)	1.29 (1.07–1.63)	1.27 (1.00–1.58)	−0.854	0.393
Low density lipoprotein (mmol/L)	3.31 ± 1.50	3.08 ± 1.21	−1.047	0.296
APTT (s)	29.62 ± 4.61	30.99 ± 5.98	1.377	0.170
INR	0.90 ± 0.086	0.92 ± 0.0936	1.527	0.128
PTA (%)	121.75 ± 22.55	111.11 ± 28.28	−2.248	**0.026**
Aspartic aminotransferase (U/L)	25.99 ± 12.64	26.19 ± 12.89	0.088	0.930
Alanine aminotransferase (U/L)	22.00 (15.85–27.70)	20.00 (15.00–25.40)	−1.236	0.217
Alkaline phosphatase (U/L)	76.19 ± 23.74	76.11 ± 55.39	−0.008	0.993
Cholinesterase (U/L)	8298.01 ± 2553.40	8536.85 ± 2523.37	0.544	0.587
γ-glutamyl transpeptidase	42.03 ± 26.91	37.91 ± 27.03	−0.880	0.380
Total protein (g/L)	69.23 ± 7.60	67.19 ± 6.59	−1.730	0.084
Albumin (g/L)	38.94 ± 9.12	38.09 ± 4.98	−0.824	0.411
Globulin (g/L)	32.79 ± 6.10	29.43 ± 4.50	−3.309	**0.002**
Random blood glucose (mmol/L)	7.88 ± 2.47	8.17 ± 4.75	0.375	0.708

APTT, activated partial thromboplastin time; INR, international normalized ratio; PTA, prothrombin time activity percentage.

Table 3. Results of logistic regression

Factors	Partial regression coefficient	Standard error	P	OR	95% CI
Globulin (g/L)	0.169	0.042	<0.001	1.185	1.090–1.288
PTA (%)	0.016	0.007	0.018	1.016	1.003–1.029
White blood cell count (10^9/L)	0.093	0.041	0.025	1.097	1.012–1.190
NIHSS score	0.092	0.031	0.003	1.097	1.031–1.166

PTA, prothrombin time activity percentage.

Table 4. Globulin levels in different HT subtypes and non-HT

Group	Globulin level (g/L)	*t*	*P* (*vs* non-HT)
HI1 (*n*=15)	30.03 ± 6.37	−0.356	0.727
HI2 (*n*=17)	32.82 ± 6.15	−2.213	0.040
PH1 (n=6)	34.75 ± 4.48	−2.847	0.005
PH2 (*n*=3)	38.77 ± 5.92	−3.549	<0.001
HI2+PH1+PH2	33.95 ± 5.90	−3.744	0.001
Non-HT group (*n*=175)	29.43 ± 4.50		

globulin level, PTA, white blood cell count, and NIHSS score, and found that all four were significantly increased in the HT group (Table 3).

In addition, we did not find any significant difference in age, sex, history of stroke, hypertension, diabetes mellitus, hyperlipidemia, coronary artery disease and atrial fibrillation, smoking, alcohol consumption, thrombolytic agent, time-window for intra-arterial thrombolysis, recanalization, NIHSS score before intra-arterial thrombolysis, systolic blood pressure before intra-arterial thrombolysis, and laboratory tests between the symptomatic HT and asymptomatic HT groups. However, compared to the non-HT group, the globulin level was significantly increased in the HI2, PH1, PH2, and HI2+PH1+PH2 groups, but not in the HI1 group (*P* = 0.727) (Table 4).

DISCUSSION

In the present study, we demonstrated that the incidence rate of HT after intra-arterial thrombolysis was 19%, similar to previous studies[1, 2]. The mortality rates were similar in the HT and non-HT groups, indicating that HT did not increase the risk of death in patients receiving intra-arterial thrombolysis. In addition, using the multifactorial logistic regression model, we found that the globulin level, PTA, white blood cell count, and NIHSS score were significantly increased in the HT group compared with the non-HT group. These results are not completely consistent with previous reports[3]. Notably, we found that the increase in globulin level was an independent risk factor for HT in patients receiving intra-arterial thrombolysis.

Previous studies have indicated that in some patients with acute ischemia, inflammatory cytokines tend to increase, and these patients tend to have a poor prognosis[10]; however, the underlying pathogenesis of the increased inflammatory cytokines is not well understood[11-13]. A recent study has identified six genes that correspond to the differences in inflammation between stroke patients with later development of HT and those without HT[13]. The differences in genetic regulation of inflammatory processes might explain why some develop a greater inflammatory response than others[10, 13, 14]. The cytokines including interleukin-6 (IL-6), interleukin-1 (IL-1), and tumor necrosis factor α (TNFα) can act on the liver and induce the synthesis of positive acute-phase reactants by the liver mediated by acute-phase protein genes[7, 11, 15-18]. Most of these are globulins, including C-reactive protein, α_2-macroglobulin, prothrombin, fibrinogen, and serum amyloid A (among others), and this might explain the increased serum globulin level in our study. Some of these acute-phase proteins (prothrombin, fibrinogen, and others) can affect the coagulation mechanism, which may also be related to HT (Fig. 1).

Simultaneously, some inflammatory cytokines, most importantly IL-1, IL-6, and TNFα, can strongly up-regulate matrix metalloproteinase 9 (MMP-9)[19-22], an independent biochemical predictor of HT in all stroke subtypes[23]. A previous study indicated that MMP-9 is involved in tPA-associated hemorrhage, and combination therapy of tPA with MMP inhibitors may help decrease the risk and severity of HT (Fig. 1)[24]. Therefore, uncontrolled MMP activation after tPA reperfusion can degrade critical proteins in the cerebrovasculature, thus disrupting vascular structural integrity and eventually leading to rupture of the vessel[24].

In this study, we assumed that the inflammatory cytokines were more active in the HT group than in the non-HT group due to genetic variation of the inflammatory system. It appears that MMP-9 could be induced by IL-1, IL-6, and TNFα, resulting in a high incidence of HT. Meanwhile, acute-phase reactants synthesized by the liver were increased, as well as the globulin level. Serum globulin electrophoresis may be useful for predicting HT in patients receiving intra-arterial thrombolysis therapy; however, because of the retrospective design of this study, we were unable to obtain data regarding inflammatory cytokines in

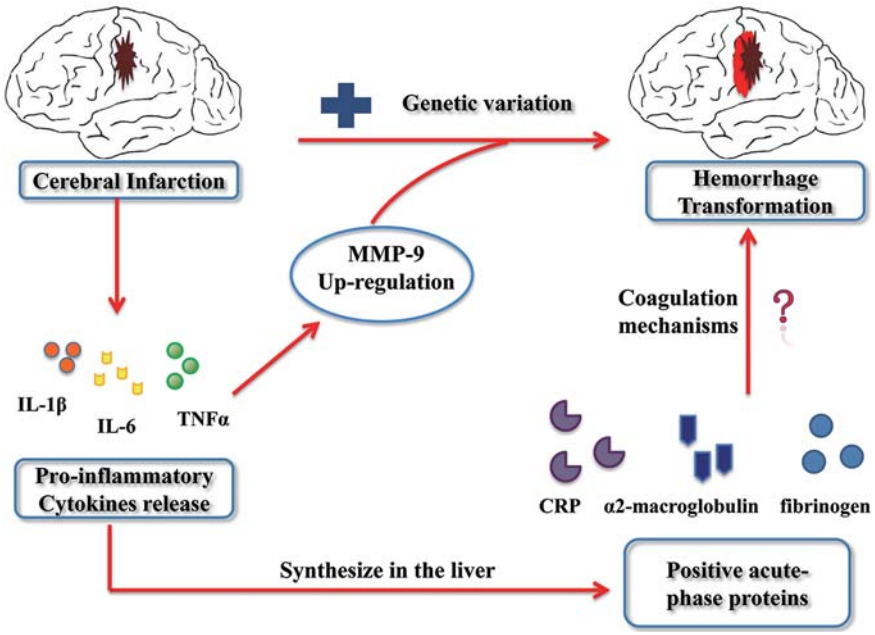

Fig. 1. Schematic of proposed hemorrhagic transformation in patients receiving intra-arterial thrombolysis. In some patients with acute ischemia, levels of inflammatory cytokines tend to be higher due to the genetic variation of the inflammatory system. MMP-9 may be induced by IL-1, IL-6, and TNFα, resulting in a high incidence of hemorrhagic transformation. In addition, synthesis of acute-phase reactants by the liver is increased, together with increased globulin level.

our patients. In fact, Jickling *et al.* have identified genes that correspond to differences in inflammation and may be a predictor of HT in ischemic stroke[13], supporting the hypothesis of an immune inflammatory response. Notably, we did not find any significant difference in globulin levels between the symptomatic and asymptomatic HT groups, probably due to the small sample size and other unknown mechanisms. We intend to perform future studies focusing on the role of cytokines in HT.

In our single-factor analysis of the risk factors for HT, the *P* value for the difference in white blood cell count between HT and non-HT groups was 0.067, close to 0.05; thus, we included white blood cell count in the subsequent multifactor logistic regression model. We found that white blood cell count was significantly increased in the HT group. The pathogenesis of the increased white blood cell count in the HT group is not well understood, but it may be associated with the stress response after cerebral infarction or the effect of acute-phase proteins. We were also unable to account for the mechanism underlying the significant difference in PTA between the two groups (even though the OR value was close to 1.0). We suspect the pathogenesis may have some association with acute-phase reactants synthesized by the liver. Some positive acute-phase proteins such as fibrinogen, prothrombin, factor VIII, and von Willebrand factor (among others) may influence the coagulation mechanism. We also found that, in the HT group, the NIHSS score before intra-arterial thrombolysis was higher, which was similar to the study by Kidwell *et al*., who indicated that a higher NIHSS score is an independent predictor of any HT[3]. Further studies are necessary to explore these mechanisms.

D'Amelio *et al.* indicated that lower levels of total cholesterol and low-density lipoprotein cholesterol are associated with HT[7], but we did not find this association. The disparity may be due to our inclusion criteria. All of our patients received intra-arterial thrombolysis therapy, but none of the patients in the study by D'Amelio *et al.* received this therapy. Other previous studies have shown that serum glucose level is an independent predictor of HT[2, 25]; controlling serum glucose level may reduce the risk of HT. However, our results did not indicate that serum glucose levels predicted HT, similar to the findings of Kimura *et al.*[26].

It also must be emphasized that the use of arterial

thrombolytic agents remains experimental. The 2003 Guidelines of the American Heart Association/American Stroke Association Stroke Council concluded that recombinant prourokinase shows some benefits in the treatment of some patients with acute ischemic stroke secondary to occlusion of the middle cerebral artery, however, the Food and Drug Administration has not approved its clinical application[27]. Extrapolation to rt-PA and urokinase, the widely-used intravenous cerebral thrombolytic agents, is based on consensus and case series data[27, 28]. In our study, we did not find any significant relationships of thrombolytic agents in the HT and non-HT groups.

Experts have been trying to find predictors in recent years and some prediction schemes have been produced. Hallevi *et al*. proposed the Houston IAT score, which is useful in comparing cohorts of patients and in assessing the results of clinical trials of HT[29]. Our study describes the risk factors for HT in patients receiving intra-arterial thrombolysis using an analysis of routine laboratory data. We found that the increased globulin level was an independent risk factor for HT in patients receiving intra-arterial thrombolysis. Furthermore, we proposed possible explanations for this phenomenon that involve inflammatory cytokines (IL-1, IL-6, and TNFα), MMP-9, and positive acute-phase reactants synthesized by the liver. We hope our findings will attract the attention of clinicians to explore the pathophysiological mechanisms behind HT. Since this is a retrospective study, some limitations such as recall bias and record bias may affect the accuracy of our data. Meanwhile, some indicators such as infarct size and the data of long-term outcome, were incomplete and hard to analyze. Although one aim of this study was to select appropriate patients for intra-arterial thrombolysis, actually the mortality rates in the HT and non-HT groups were similar, and coupled with the lack of data of long-term outcome, the aim could not be achieved. It is also worth mentioning that different subtypes of HT (HI1, HI2, PH1, and PH2) may have different pathophysiological mechanisms, predictors, and outcomes. For example, Molina *et al*. reported that HI (HI1-HI2) may indicate early successful recanalization and better outcome[5]. In our study, we found that globulin level was significantly increased in some subtypes (HI2, PH1, and PH2) compared to the non-HT group. Nevertheless, we have to admit that our sample size is too small. Further studies are necessary to explore predictors for different subtypes of HT.

In conclusion, an increase in globulin level is an independent risk factor for HT in patients receiving intra-arterial thrombolysis, with the possible mechanisms involving inflammatory cytokines (IL-1, IL-6, and TNFα), MMP-9, and positive acute-phase reactants synthesized by the liver. Serum globulin electrophoresis may be useful for predicting HT in this population.

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