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Original Article

Correlation between the levels of NLRP3, Hcy, IL-1β, IL-18 and the prognosis in patients with hemorrhagic stroke

Qingyong Li[1*], Zhiheng Zhao[2*], Kun Si[1], Jianguo Zhou[1], Li'na Du[1], Hua Liu[1], Ru Lin[3]

[1]Department of Neurosurgery, Shengli Hospital of Dongying, Dongying, Shandong Province, China; Departments of [2]Neurology, [3]Blood Transfusion, Shengli Oilfield Central Hospital, Dongying, Shandong Province, China. [*]Equal contributors and co-first authors.

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Abstract: Objective: To explore the connection of nucleotide-binding oligomerization domain-like receptors 3 (NLRP3), homocysteine (Hcy), interleukin-1β (IL-1β), interleukin-18 (IL-18) in peripheral blood and prognosis in patients with hemorrhagic stroke. Methods: A total of 84 patients with hemorrhagic stroke treated in our hospital were selected and divided into the good prognosis group (48 cases) and the poor prognosis group (36 cases) according to the Glasgow Prognostic Scale (GOS) at month 6 after discharge. 40 people who were matched for age, sex and risk factors for cerebral hemorrhage, but did not have cerebral hemorrhage, were selected as a control group. We detected the levels of NLRP3, Hcy, IL-1β and IL-18 in peripheral blood, and analyzed their correlation with GOS score. Then we performed Logistic regression analysis to investigate the risk factors for poor prognosis. Results: The expressions of NLRP3 mRNA, Hcy, IL-1β and IL-18 in peripheral blood in the poor prognosis group were higher than those in the good prognosis group ($P<0.05$). The expression levels of NLRP3 mRNA, Hcy, IL-1β and IL-18 were negatively correlated with GOS scores ($P<0.05$). Regression analysis showed that the expression of NLRP3 mRNA, serum Hcy, bleeding volume and ventricular system penetration were independent risk factors for poor prognosis. Conclusion: In patients with poor prognosis of hemorrhagic stroke, the mRNA levels of NLRP3 and serum Hcy, IL-1β and IL-18 levels in peripheral blood elevated. High NLRP3 mRNA levels, Hcy levels, bleeding volume and ventricle system penetration are independent risk factors for poor prognosis.

Keywords: Hemorrhagic stroke, nucleotide-binding oligomerization domain-like receptors 3, inflammasome, homocysteine, interleukin-1β, interleukin-18

Introduction

Stroke is a disease with extremely high rate of disability and fatality. According to the 2016 Global Burden of Disease, there are 13.7 million new stroke patients worldwide, among which China accounts for 40%. Moreover, 5.5 million people die from stroke, among which China accounts for about 33% [1]. Stroke is divided into ischemic stroke and hemorrhagic stroke. Hemorrhagic stroke accounts for about 20%-30% of stroke patients [2]. Hemorrhagic stroke refers to non-traumatic spontaneous hemorrhage in the brain parenchyma, including subarachnoid hemorrhage and cerebral hemorrhage [3]. Hemorrhagic stroke develops rapidly, reaching its peak within a few minutes to several hours. In addition to typical symptoms such as severe headache, nausea, vomiting, restlessness, drowsiness, and coma, patients often have increased intracranial pressure, brain herniation, breathing failure, impaired consciousness immunosuppression and often require invasive operations such as surgery and assisted ventilation. Hemorrhagic stroke poses a great threat to patients' life [4-6]. Therefore, it is of great value to search for indicators that can effectively reflect the severity and prognosis of patients with hemorrhagic stroke. Homocysteine (Hcy) is a sulfur-containing amino acid and an intermediate product of methionine metabolism. It was found that the abnormality of Hcy is highly related to the occurrence of cardiovascular disease [7]. Nucleotide-

binding oligomerization domain-like receptors 3 (NLRP3) in peripheral blood mononuclear cells (PBMCs) is a polyprotein complex present in cytoplasm, whose role includes regulating the secretion of a variety of inflammatory factors such as interleukin-1β (IL-1β) and interleukin-18 (IL-18) etc. [8]. In recent years, multiple studies have shown that NLRP3, IL-1β, and IL-18 are closely related to the occurrence and development of cerebral hemorrhagic disease, but there are few studies on their correlation with cerebral hemorrhage prognosis [9]. In this study, we explored the relationship between levels of NLRP3, IL-1β, IL-18 in peripheral blood and prognosis in patients with hemorrhagic stroke, with the aim of providing theoretical basis for clinical prediction of patient prognosis.

Materials and methods

General materials

This study was approved by the Ethics Committee of Shengli Hospital of Dongying, and the patient's families or guardians signed the informed consent. A total of 84 patients with hemorrhagic stroke who were treated in Shengli Hospital of Dongying from January 2018 to January 2019 were selected as research objects and set as the research group. The patients were followed up for 6 months after discharge and the Glasgow Outcome Scale (GOS) was used for evaluation of prognosis at the sixth month after discharge [10]. The highest score is 5, and the lower the score is, the worse the prognosis is. 5 points indicated mild defect but good recovery and a normal life; 4 points indicated mild disability and independence; 3 points indicated severe disability and life needs others to take care; 2 points indicated survival in a vegetative state; 1 point indicated death. We divide them into a good prognosis group (GOS score of 4 to 5) of 48 cases and a poor prognosis group (GOS score of 1 to 3) of 36 cases. At the same time, we recruited 40 patients who came to the physical examination center of Shengli Hospital of Dongying for physical examination without a history of cerebral hemorrhage as the control group. The diagnostic criteria for cerebral hemorrhage referred to the diagnostic criteria for hemorrhagic stroke in the "China Guidelines for the Diagnosis and Treatment of Cerebral Hemorrhage" formulated by the Chinese Medical Association Neurology Branch in 2014 [11].

Inclusion criteria: (1) Patients who met the above diagnostic criteria and were confirmed to be hemorrhagic stroke by brain CT or MRI; (2) Patients who were admitted to the hospital within 12 hours of onset; (3) Patients treatment naive before admission to the hospital.

Exclusion criteria: (1) Patients with cerebral hemorrhage caused by other causes such as cerebral infarction, intracranial artery rupture, trauma, blood disease, drugs, etc.; (2) Patients with severe infection; (3) Patients with severe organ dysfunction; (4) Patients with history of mental disorder; (5) Patients with malignant tumor.

Methods

Data collection: We collected clinical data of patients, including patients' age, gender, smoking history, body mass index (BMI), history of hypertension, history of diabetes, comorbidity of hyperlipidemia, smoking/alcohol use, vital signs on admission (respiration, pulse, blood pressure, heart rate) and general laboratory tests (routine blood tests, coagulation tests, biochemistry), GOS score at 6 months after discharge, etc.

Measurement of the mRNA expression of NLRP3 in peripheral blood mononuclear cells: We collected 6.0 mL of fasting peripheral venous blood from patients who did not undergo surgery in the morning after admission, and 6.0 mL of fasting peripheral venous blood was collected from patients who received surgeries in the morning after surgeries. The expression of NLRP3 mRNA in monocytes in peripheral blood was measured by RT-PCR. The specific operation was as follows: (1) After separating plasma, we extracted PBMCs in peripheral blood by Ficoll-Hypaque density separation, and extracted total RNA according to the instructions of E.Z.N.A DNA/RNA/Protein Isolation Kit (R-6734-01, Shanghai Suobao Biotechnology Co., Ltd., China). (2) We took quantitative RNA (1.0 g) for reverse transcription to synthesize cDNA. The RT process was as follows: reaction at room temperature for 10 min and reaction at 42°C for 30 min, and reverse transcriptase at 95°C for 5 min. (3) PCR amplification: we designed primers and

Table 1. NLRP3 mRNA & intrinsic parameter GAPDH order

Gene	Forward primer 5'-3'	Reverse primer 3'-5'
NLRP3 mRNA	CTTCCTTTCCAGTTTGCTGC	TCTCGCAGTCCACTTCCTTT
GAPDH	CTTCTCTGATGAGGCCCAAG	GCAGCAAACTGGAAAGGAAG

Note: NLRP3: nucleotide-binding oligomerization domain-like receptors 3.

selected internal reference (**Table 1**), and performed translation in the PCR system under the following conditions: reaction at 94°C for 1 min, denaturation for 20 s, reaction at 56°C for 1.5 min, reaction at 72°C for 2 min, cycling for 30 times, and then extending for 10 min at 72°C. (4) Each test was repeated for 3 times, and the expression of NLRP3 mRNA was calculated by the $2^{-\Delta\Delta Ct}$ method.

Determination of serum Hcy, IL-1β and IL-18 levels: 10.0 mL of fasting peripheral venous blood was collected from patients who did not undergo surgery in the morning after admission, and 10.0 mL of fasting peripheral venous blood was collected from patients undergoing surgery in the morning after surgery. Both were placed in a disposable anticoagulation tube and centrifuged at 3000 r/min for 10 min. Then we took the supernatant, and kept it in a refrigerator at -80°C for testing. We adopted fluorescence polarization immunoassay to take the detection of Hcy and used OP-162 microfluorescence detector (The matching reagents were purchased from Shandong Bomaida Biotechnology Co., Ltd., China); IL-1β and IL-18 were determined by enzyme-linked adsorption method (ELISA), and the ELISA kits used were purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd., China.

Outcome measures

Main outcome measures: (1) Expression differences in NLRP3, Hcy, IL-1β, and IL-18 levels in peripheral blood in patients with different prognosis; (2) Related risk factors for poor prognosis in patients with hemorrhagic stroke.

Secondary observation indicators: (1) The correlation between the expression of NLRP3 in PBMCs and serum Hcy, IL-1β, and IL-18 in peripheral blood of patients with hemorrhagic stroke and GOS score; (2) The influences of bleeding volume, bleeding sites and treatment methods on theprognosis of hemorrhagic stroke patients.

Statistical analysis

SPSS 26.0 software was used for statistical analysis, the measurement data was expressed in the form of mean ± standard deviation ($\bar{x}$ ± sd), and the count data were expressed in percentage. The comparison between groups was performed with t test or χ^2 test; Pearson correlation analysis was used to analyze the correlation between NLRP3 mRNA expression and serum Hcy, IL-1β and IL-18 levels and the GOS score of the patient's prognosis; Multivariate logistic multivariate regression analysis was used to analyze the relevant factors affecting prognosis of patients. P<0.05 was considered statistically significant.

Results

Comparison between the study group and the control group

The study group contained a total of 84 cases and the control group contained a total of 40 subjects. There was no difference in age, gender, BMI, smoking, drinking, whether combined with hypertension, diabetes, hypercholesterolemia and (all P>0.05). The expression of NLRP3 mRNA and Hcy, IL-1β, and IL-18 levels in peripheral blood monocytes of the study group were significantly higher than those of the control group (all P<0.001). See **Tables 2**, **3**.

Comparison of the general data of the two groups of patients with different prognosis

There were 48 cases in the good prognosis group and 36 cases in the poor prognosis group. There were no difference in age, gender, BMI, smoking, drinking, bleeding sites, whether combined with hypertension, diabetes, hypercholesterolemia and cardiovascular disease (all P>0.05). The bleeding volume and the number of people with hemorrhage broking into the ventricle in the poor prognosis group were higher than those in the good prognosis group (P<0.05).

NLRP3 mRNA expression in peripheral blood mononuclear cells and serum Hcy, IL-1β, and IL-18 concentrations were higher in the poor prognosis group than those in the good prognosis group. NLRP3 mRNA expression in periph-

Table 2. Comparison of general data of the study group and the control group

Item	Study group (n = 84)	Control group (n = 40)	χ^2/t	P
Age	63.4±6.3	61.6±5.4	0.449	0.124
Gender (Male/Female)	48/36	28/12	1.888	0.169
BMI (kg/m^2)	25.62±2.25	25.31±2.15	0.357	0.459
Smoking	58	26	0.203	0.652
Drinking	53	24	0.110	0.740
Whether combined with hypertension	56	29	0.428	0.513
Whether combined with diabetes	38	16	0.302	0.582
Whether combined with hypercholesterolemia	55	28	0.251	0.617
Whether combined with cardiovascular disease	42	16	1.703	0.427

Note: BMI: body mass index.

Table 3. Comparison of the expression of NLRP3 mRNA, serum Hcy, IL-1β, and IL-18 levels of the study group and the control group

Group	NLRP3 mRNA	Hcy (umol/L)	IL-1β (pg/mL)	IL-18 (ng/L)
Study group (n = 84)	1.59±0.32	33.74±17.34	21.13±4.91	68.75±12.86
Control group (n = 40)	0.93±0.28	22.93±15.95	15.67±12.86	49.79±14.15
t	0.340	2.322	0.881	0.582
P	<0.001	<0.001	<0.001	<0.001

Note: Hcy: homocysteine; IL-1β: interleukin-1β; IL-18: interleukin-18; NLRP3: nucleotide-binding oligomerization domain-like receptors 3.

Table 4. Comparison of the general data and NLRP3 mRNA expression, serum Hcy, IL-1β, IL-18 of the good prognosis group and the poor prognosis group

Item	Good prognosis group (n = 48)	Poor prognosis group (n = 36)	χ^2/t	P
Age (year)	63.1±6.8	63.9±5.7	3.177	0.568
Gender (male/female)	28/20	20/16	0.065	0.799
BMI	25.81±2.32	25.37±2.14	1.090	0.375
Smoking	36	22	3.384	0.066
Drinking	27	26	2.254	0.133
Whether combined with hypertension	34	22	0.875	0.350
Whether combined with diabetes	18	20	2.707	0.100
Whether combined with hypercholesterolemia	33	22	0.531	0.466
Whether combined with cardiovascular disease	20	22	3.576	0.167
Hemorrhage site (cortex/basal ganglia/thalamus/cerebellum	8/33/6/1	1/24/9/2	6.211	0.102
The number of people broke into the ventricle	20	26	7.753	0.005
Operative treatment	32	27	0.297	0.586
Bleeding volume (mL)	31.45±6.85	41.98±6.80	0.103	<0.001
NLRP3 mrna	1.45±0.31	1.77±0.22	3.003	<0.001
Hcy (umol/L)	27.79±14.51	41.68±17.80	3.757	<0.001
IL-1β (pg/mL)	20.13±4.33	22.46±5.37	2.295	0.031
IL-18 (ng/L)	65.66±11.21	72.87±13.89	0.244	0.010

Note: Hcy: homocysteine; IL-1β: interleukin-1β; IL-18: interleukin-18; NLRP3: nucleotide-binding oligomerization domain-like receptors 3.

eral blood mononuclear cells and serum Hcy in the poor prognosis group were significantly higher than those in the good prognosis group (P<0.001). See **Table 4**.

Table 5. The correlation of NLRP3 mRNA expression and serum Hcy, IL-1β, IL-18 levels in monocytes in peripheral blood and the prognostic GOS score of patients with hemorrhagic stroke

Factor	Relativity r	P
NLRP3 mRNA	-0.480	<0.001
Hcy	-0.390	<0.001
IL-1β	-0.221	0.044
IL-18	-0.224	0.041

Note: Hcy: homocysteine; IL-1β: interleukin-1β; IL-18: interleukin-18; NLRP3: nucleotide-binding oligomerization domain-like receptors 3; GOS: glasgow outcome scale.

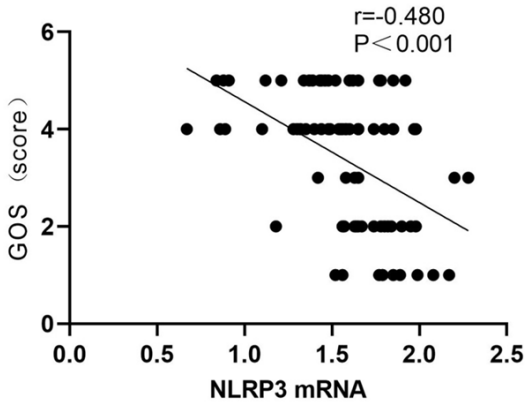

Figure 1. The correlation of NLRP3 mRNA expression and the GOS score mRNA. NLRP3: nucleotide-binding oligomerization domain-like receptors 3; GOS: glasgow outcome scale.

The correlation of NLRP3 mRNA expression in peripheral blood mononuclear cells and serum Hcy, IL-1β, IL-18 levels and the prognostic GOS score of patients with hemorrhagic stroke

The NLRP3 mRNA expression and serum Hcy, IL-1β, IL-18 levels in monocytes in peripheral blood was negatively correlated with the prognostic GOS score of patients with hemorrhagic stroke (all P<0.05). See **Table 5** and **Figures 1**-**4**.

Logistic regression analysis of adverse factors affecting the prognosis of patients with hemorrhagic stroke

We took the prognosis of patients with hemorrhagic stroke as the dependent variables (0 = good prognosis, 1 = bad prognosis), and the expression of NLRP3 mRNA and serum Hcy, IL-1β, IL-18, bleeding volume, and whether the ventricle was broken into of the diseased group were independent variables. The above variables that may lead to poor prognosis were assigned, and binary Logistic regression analysis was performed. The results showed that the expression of NLRP3 mRNA in peripheral blood monocytes was ≥1.63, and serum Hcy ≥21.5 umol/L, hemorrhage breaking into the ventricle, and bleeding volume >35 mL are independent risk factors for poor prognosis in patients with hemorrhagic stroke. See **Tables 6**, **7**.

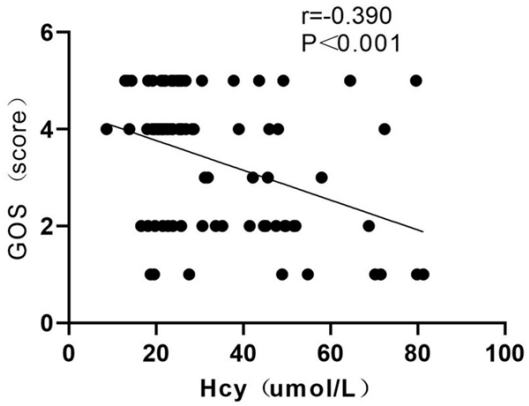

Figure 2. The correlation of serum Hcy and the GOS score. Hcy: homocysteine; GOS: glasgow outcome scale.

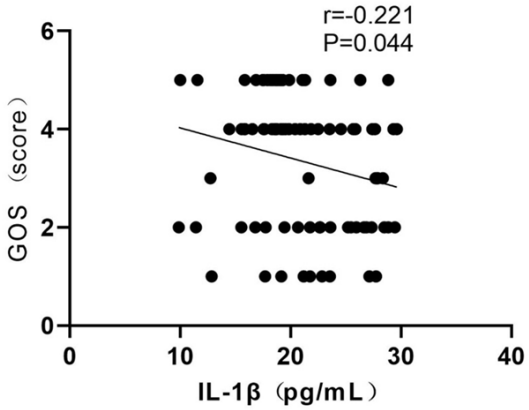

Figure 3. The correlation of serum IL-1β and the GOS score. IL-1β: interleukin-1β; GOS: glasgow outcome scale.

Discussion

Hcy is a type of amino acid with sulfur-containing molecules, which is generated by methionine demethylation in vivo. The process requires the participation of methionine synthetase, cystathionine β synthase, vitamin B12,

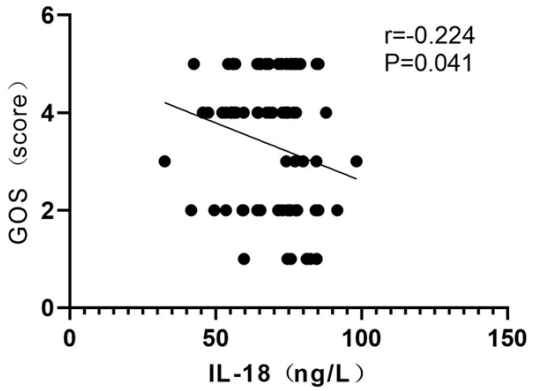

Figure 4. The correlation of serum IL-18 and the GOS score. IL-18: interleukin-18; GOS: glasgow outcome scale.

folic acid and vitamin B6. Current studies have shown that high concentration of Hcy is an independent risk factor for coronary artery disease, ischemic cerebrovascular disease and peripheral vascular ischemic disease [12]. However, there are only few studies which clarify the relationship between Hcy elevation and hemorrhagic stroke. Cerebral parenchymal hemorrhage accounts for the vast majority of hemorrhagic stroke. There are several reasons for vascular endothelial damage in current studies. Due to various factors, hyaline change and fibronecrosis occur in the small artery wall, and the elasticity of the wall is weakened, making it easy to rupture and bleed [13]. While the increase of Hcy will cause endothelial cell hypertrophy and damage. This leads to rupture of vascular elastic membrane, hypertrophy and damage of middle smooth muscle cells, increase of interstitial collagen fibers, and hardening of vascular wall, which leads to increased blood pressure [14]. Hypertension is one of the most common causes of hemorrhagic stroke [15]. At the same time, weak tube wall under the impact of blood flow is also prone to pathological changes, resulting in various sizes of aneurysms, which leads to cerebral parenchymal hemorrhage after rupture [16]. Meanwhile, the increase of Hcy also has cytotoxic effect and stimulates LDL oxidation [17, 18].

NLRP3 is a member of the intracellular nucleotide binding oligomeric domain like receptors (NLRs) family. A current study has confirmed that NLRP3 is widely involved in the process of immune and inflammatory response [8]. NLRP3 is related to the maturation and secretion of pro-inflammatory cytokines IL-1β and IL-18. NLRP3 is widely distributed in the cytoplasmic receptor proteins. Once the danger signal is recognized, the protein structure of NLRP3 transfers, which can form protein composites, that is, inflammatory corpuscle, with the adapter protein (ACS) and the effect protein, Procaspase I, to induce programmed cell death [19, 20]. Many studies have shown that NLRP3 and IL-1β, IL-18 produced by NLRP3 are closely related to the occurrence and development of various diseases such as diabetes, nervous system diseases and infectious diseases [21-23]. Inflammatory cytokines such as IL-1β can activate the exogenous coagulation system, and then lead to the formation of vascular endothelial thrombus, causing serious inflammatory reaction, damaging the vascular wall and leading to cerebral hemorrhage [24]. It was proved that inflammatory response is closely related to acute brain injury and prognosis of cerebral hemorrhage [25].

In this study, the expression of NLRP3 mRNA in peripheral blood mononuclear cells and the levels of serum Hcy, IL-1β and IL-18 of patients with hemorrhagic stroke in poor prognosis group were significantly higher than those in good prognosis group, and the four were negatively correlated with prognosis GOS score. Logistic regression analysis showed that NLRP3 mRNA expression in peripheral blood mononuclear cells and serum Hcy, IL-1β and IL-18 levels were significantly higher than those in good prognosis group mRNA expression, serum Hcy level, blood loss and ventricular rupture were independent risk factors for poor prognosis in patients with hemorrhagic stroke. Previous studies on the correlation between NLRP3 and cerebral hemorrhage were mostly from animal experiments [26]. There were few studies on clinical prognosis. The results of this study have certain clinical significance.

However, there are still several drawbacks in this study. Due to the relatively small number of cases included, NLRP3 and Hcy, IL-1β, IL-18 were obtained from peripheral blood mononuclear cells, and there were no other body fluid or cytology related samples (it was reported that the level of NLRP3 in cerebrospinal fluid of infants after brain injury increased) for comparison research [27]. At the same time, the fol-

Table 6. Assignment of independent variables by type for influencing factors on the prognosis of patients with hemorrhagic stroke

Factor	Independent variable	Assignment
NLRP3 mRNA expression	X1	<1.63 = 0, ≥1.63 = 1
Hcy (umol/L)	X2	<21.5 umol/L = 0, ≥21.5 umol/L = 1
IL-1β (pg/mL)	X3	<20.78 pg/mL = 0, ≥20.78 pg/mL = 1
IL-18 (ng/L)	X4	<72.76 ng/L = 0, ≥72.76 ng/L = 1
Bleeding volume (mL)	X5	≤35 mL = 0, >35 mL = 1
Whether broke into the ventricle	X6	Yes = 1, No = 0

Note: Hcy: homocysteine; IL-1β: interleukin-1β; IL-18: interleukin-18; NLRP3: nucleotide-binding oligomerization domain-like receptors 3.

Table 7. Logistic regression analysis of adverse factors affecting the prognosis of patients with hemorrhagic stroke

Factor	Regression coefficients	Standard error	Wald value	OR value	95% CI	P
NLRP3 mRNA	0.813	0.244	11.122	2.254	1.398-3.634	<0.001
Hcy	0.082	0.031	6.781	1.085	1.017-1.154	0.009
IL-1β	0.156	0.102	2.331	1.169	0.957-1.427	0.127
IL-18	0.057	0.043	1.829	1.059	0.975-1.151	0.176
Bleeding volume (mL)	0.325	0.088	13.576	1.384	1.164-1.646	<0.001
Whether broke into the ventricle	1.292	0.473	7.446	3.640	1.439-9.207	0.006

Note: Hcy: homocysteine; IL-1β: interleukin-1β; IL-18: interleukin-18; NLRP3: nucleotide-binding oligomerization domain-like receptors 3.

low-up period was too short to observe the long-term prognosis, which may affect the final results and lead to bias. In order to improve the accuracy of the study, the sample size should be enlarged for further study.

Disclosure of conflict of interest

None.

Address correspondence to: Ru Lin, Department of Blood Transfusion, Shengli Oilfield Central Hospital, No. 31 Ji'nan Road, Dongying 257034, Shandong Province, China. Tel: +86-18554675308; E-mail: linru51sl@163.com

References

[1] Zhang XT, Zhou Y, Zhang S, Ding WH and Lou M. Cerebral blood flow evaluation of intensive rosuvastatin therapy in stroke/transient ischemic attack patients with intracranial arterial atherosclerotic stenosis study: rationale and design. Brain Behav 2017; 7: e00689.

[2] Frontera JA, Starling R, Cho SM, Nowacki AS, Uchino K, Hussain MS, Mountis M and Moazami N. Risk factors, mortality and timing of ischemic and hemorrhagic stroke with left ventricular assist devices. J Heart Lung Transplant 2016; 36: 673-683.

[3] Chen LC, Chen MH, Su TP, Tsai S, Bai YM, Li CT, Yang AC, Chang WH and Chen TJ. Atopic diseases/diathesis and subsequent ischemic stroke among patients with bipolar disorder: a nationwide longitudinal study. PLoS One 2018; 13: e0200682.

[4] Al-Khaled M and Eggers J. Prognosis of intracerebral hemorrhage after conservative treatment. J Stroke Cerebrovasc Dis 2014; 23: 230-234.

[5] Archana H, Jamil D, Eugene A, Patel A, Samant R and Yaghi S. Nosocomial infections in patients with spontaneous intracerebral hemorrhage. Am J Crit Care 2015; 24: 227-231.

[6] Chen GF, Ping L, Zhou SK, Liu WW, Liu LJ, Zhang DM, Li ZL, Tian YF and Chen Z. Early prediction of death in acute hypertensive intracerebral hemorrhage. Exp Ther Med 2016; 11: 83-88.

[7] Kalam AMA, Pan H, Gang L, Ren WK, Teklebrh T, Yan WX, Zhou XH and Yin YL. Hyperhomocysteinemia and cardiovascular disease in animal model. Amino Acids 2018; 50: 3-9.

[8] Pérez-Figueroa E, Torres J, Sánchez-Zauco N, Contreras-Ramos A, Alvarez-Arellano L and Maldonado-Bernal C. Activation of NLRP3 inflammasome in human neutrophils by helico-

bacter pylori infection. Innate Immun 2016; 22: 103-112.
[9] Yang XL, Sun J, Kim TJ, Kim Y, Ko S, Kim CK, Jia XF and Yoon B. Pretreatment with low-dose fimasartan ameliorates NLRP3 inflammasome-mediated neuroinflammation and brain injury after intracerebral hemorrhage. Exp Neurol 2018; 310: 22-32.
[10] Fuller GW, Hernandez M, Pallot D, Lecky F, Stevenson M and Gabbe B. Health state preference weights for the glasgow outcome scale following traumatic brain injury: a systematic review and mapping study. Value Health 2016; 20: 141-151.
[11] Chinese Medical Association Neurology Branch and Chinese Medical Association Neurology Branch Cerebrovascular Disease Group. Guidelines for the diagnosis and treatment of cerebral hemorrhage in China (2014). Chin J Neurol 2015; 48.
[12] Wang Y, Zhang J, Qian YS, Tang XF, Ling HW, Chen KM, Li Y, Gao PJ and Zhu DL. Association of homocysteine with aysmptomatic intracranial and extracranial arterial stenosis in hypertension patients. Sci Rep 2018; 8: 595.
[13] Chen S, Dong ZP, Cheng M, Zhao YQ, Wang MY, Sai N, Wang X, Liu H, Huang GW and Zhang XM. Homocysteine exaggerates microglia activation and neuroinflammation through microglia localized STAT3 overactivation following ischemic stroke. J Neuroinflammation 2017; 14: 187.
[14] Ars CL, Nijs IM, Marroun HE, Muetzel R, Schmidt M, Steenweg-de Graaff J, van der Lugt A, Jaddoe VW, Hofman A, Steegers EA, Verhulst FC, Tiemeier H and White T. Prenatal folate, homocysteine and vitamin B12 levels and child brain volumes, cognitive development and psychological functioning: the generation R study. Br J Nutr 2019; 122: S1-S9.
[15] Yang G and Shao GF. Clinical effect of minimally invasive intracranial hematoma in treating hypertensive cerebral hemorrhage. Pak J Med Sci 2016; 32: 677-681.
[16] Kiran DM, Matta RA and Lakshmana KN. Role of anthropometric measurements in development of CVD and Stroke among T2DM in East Godavari district, Andhra Pradesh, India. J Clin Diagn Res 2017; 11: BC01-BC05.
[17] Ye ZS, Zhang ZZ, Zhang H, Hao YG, Zhang J, Liu WH, Xu GL and Liu XF. Prognostic value of C-reactive protein and homocysteine in large-artery atherosclerotic stroke: a prospective observational study. J Stroke Cerebrovasc Dis 2017; 26: 618-626.
[18] Jiang YH, Guo JH, Wu S and Yang CH. Vascular protective effects of aqueous extracts of tribulus terrestris on hypertensive endothelial injury. Chin J Nat Med 2017; 15: 606-614.
[19] Li SJ, Wang M, Ojcius DM, Zhou BJ, Hu WL, Liu Y, Ma Q, Tang GP, Wang DM and Yan J. Leptospira interrogans infection leads to IL-1B and IL-18 secretion from a human macrophage cell line through reactive oxygen species and cathepsin B mediated-NLRP3 inflammasome activation. Microbes Infect 2018; 20: 254-260.
[20] Yuan RR, Fan HY, Cheng SQ, Gao WW, Xu X, Lv SG, Ye MH, Wu MJ, Zhu XG and Zhang Y. Silymarin prevents NLRP3 inflammasome activation and protects against intracerebral hemorrhage. Biomed Pharmacother 2017; 93: 308-315.
[21] Diamanti AP, Markovic M, Argento G, Giovagnoli S, Ricci A, Laganà B and D'Amelio R. Therapeutic management of patients with rheumatoid arthritis and associated interstitial lung disease: case report and literature review. Ther Adv Res Dis 2017; 11: 64-72.
[22] Dam K, Soo-Kyung C, Chan-Bum C, Choe J, Chung WT, Hong S, Jun J, Jung YO, Kim T, Kim T, Lee H, Lee J, Lee J, Lee S, Yoo D, Yoon BY, Song JW, Bae S and Sung Y. Impact of interstitial lung disease on mortality of patients with rheumatoid arthritis. Rheumatol Int 2017; 37: 1735-1745.
[23] Alf K, Lisbeth Ä and Solbritt RD. Genetic variants of the NLRP3 inflammasome are associated with stroke in patients with rheumatoid arthritis. J Rheumatol 2015; 42: 1740-1745.
[24] Adams HP Jr. Cerebral vasculitis. Handb Clin Neurol 2014; 119: 475-494.
[25] Wang Z, Zhou F, Dou Y, Tian XD, Liu CL, Li HY, Shen HT and Chen G. Melatonin alleviates intracerebral hemorrhage-induced secondary brain injury in rats via suppressing apoptosis, inflammation, oxidative stress, DNA damage, and mitochondria injury. Translat Stroke Res 2018; 9: 74-91.
[26] Wang L, Zheng SY, Zhang L, Xiao H, Gan H, Chen H, Zhai X, Liang P, Zhao J and Li YL. Histone deacetylation 10 alleviates inflammation after intracerebral hemorrhage via the PTPN22/NLRP3 pathway in rats. Neuroscience 2020; 432: 247-259.
[27] Wallisch JS, Simon DW, Bayır H, Bell MJ, Kochanek PM and Clark RSB. Cerebrospinal fluid NLRP3 is increased after severe traumatic brain injury in infants and children. Neurocrit Care 2017; 27: 44-50.