CHANGES OF HA AND NLR IN PERIPHERAL BLOOD AFTER ONSET OF ACUTE SPONTANEOUS CEREBRAL HEMORRHAGE AND THE VALUE OF COMBINED DETECTION IN EVALUATING PROGNOSIS OF PATIENTS

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ABSTRACT

Objective: *To observe the changes of hyaluronic acid (HA) and neutrophil/lymphocyte ratio (NLR) in peripheral blood after acute spontaneous intracerebral hemorrhage (AICH) and the value of combined detection in evaluating the patient prognosis.*

Methods: *102 patients with spontaneous intracerebral hemorrhage (sICH)admitted to our hospital from May 2015 to March 2017 were selected as the observation group. According to the Rankin Revised Scale, 63 patients with a score less than 3 (including 3 points) were the group with good prognosis, and 39 patients with a score greater than 3 were the group with poor prognosis. 100 normal subjects were selected as the control group. The National Institutes of Health Stroke Scale and Glasgow Coma Index Scale were used to evaluate the treatment of patients with poor prognosis and good prognosis. Serum HA levels were detected by ELISA, neutrophils and lymphocytes were detected by flow cytometry, and NIR levels were calculated. We compared the levels of HA and NLR in peripheral blood of each group. Logistic regression analysis was used to evaluate the relationship between HA and NLR in peripheral blood and prognosis of patients with sICH. The prognostic value of HA and NLR in patients with sICH was evaluated by ROC curve analysis.*

Results: *The levels of HA and NLR in the group with poor/good prognosis were higher than control group, meanwhile those in the group with good prognosis were lower than poor prognosis group (P<0.05). The NIHSS score in the group with good prognosis was lower than poor prognosis group, and the GCS score was higher than poor prognosis group (P<0.05). The average volume of hematoma in the good prognosis group was 56.79+8.67 mL, which was smaller than that in the poor prognosis group (91.28+15.96 mL) (P<0.05). Logistic regression model results showed that NIHSS score, GCS score, HA and NLR were significantly correlated with prognosis (P<0.05). The detection of HA and NLR in peripheral blood alone or in combination has high diagnostic value in evaluating the prognosis of patients with sICH.*

Conclusion: *The levels of HA and NLR in patients with sICH are raised than those in healthy people, and those may be a poor prognostic factor in patients with acute sICH. The detection of HA and NLR in peripheral blood alone or in combination has high diagnostic value in evaluating the prognosis of patients with sICH.*

Keywords: *HA, NLR, acute spontaneous intracerebral hemorrhage, prognosis.*

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Introduction

Spontaneous intracerebral hemorrhage (sICH) caused by various circumstances without the exclusion of trauma, with a high mortality and disability rate[1]. Studies have found that the fatality rate of spontaneous intracerebral hemorrhage in European and American countries is 24%~53%, among which the acute mortality rate is 32%~41%, which has a serious impact on people's physical and mental health.Hyaluronic acid (HA) and Neutrophils Lymphocyte Ratio (NLR) in peripheral blood are abnormal when patients suffer from SPONTANEOUS intracerebral hemorrhage, which are significantly related to the sICH patient's prognosis[2]. Peripheral blood HA, also known as hyaluronic acid, is an acidic mucopolysaccharide that is widely present in plasma and blood vessel wall and is involved in various physiological activities such as cell mitosis, wound repair, and endogenous

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immune activation[3]. NLR is a newly discovered inflammatory marker, which can be used as one of the evaluation indicators of inflammatory response in the acute stage of vascular injury[4]. This study mainly analyzed the changes of HA and NLR in peripheral blood in acute spontaneous intracerebral hemorrhage, and explored the value of combined HA and NLR detection in patients' prognosis.

Data and methods

General information

A total of 102 patients withsICH admitted to our hospital from May 2015 to March 2017 were selected as the observation group.

Inclusion criteria:

• All patients met the diagnostic criteria revised by the National Cerebrovascular Academic Conference[5], and were diagnosed with spontaneous intracerebral hemorrhage by head CT or Magnetic resonance imaging (MRI);

• The patient had the first onset and the onset time was less than 24 hours;

• The patient's mental symptoms are normal and can cooperate with treatment;

• The amount of blood loss is less than 40ml.

Exclusion criteria:

• Patients with severe kidney, liver, and heart dysfunction;

• People with a history of drug allergy;

• Patients who were treated with anticoagulant drugs within 8 weeks before the treatment;

• Minors or over 85 years old.

No significant differences were found in age, sex, and other basic data between the observation group and the control group (P>0.05), but significant differences were found in hypertension, smoking history, and other general data (P<0.05). See Table 1.

Group	n	Age (year)	Gender (n)		Time of onset (h)	Smoking history (n, %)	Hypertension (n, %)
			Man	Woman			
Control group	100	56.92±6.61	52	48	8.21±4.05	33 (33.00)	25 (25.00)
Observation group	102	58.69±6.24	57	45	8.67±3.91	61 (59.81)	68 (66.67)
t/χ^2		1.957	0.306		0.821	14.581	35.287
P		0.052	0.579		0.412	<0.001	<0.001

Table 1: Basic data between the two groups.

Observation indicators

The National Institute of Health Stroke Scale and Glasgow Coma Scale are used to evaluate the treatment of patients in the poor/good prognosis group.

Hematoma volume:

The ABC/2 method proposed by Kothari et al was used to measure the volume of hematoma.Where, A is the maximum diameter of the largest layer of the bleeding area; B is the maximum diameter perpendicular to A; C is the product of the number of bleeding layers multiplied by the thickness of each layer.

Peripheral blood HA and NLR detection:

5ml fasting venous blood was taken, and centrifuged for supernatant.The serum HA level was determined by Nzyme linked immunoadsorption analysis (ELISA), the content of neutrophils and lymphocytes was determined by flow cytometry, and the NIR level was calculated.

Prognosis:

Patients were followed up 3 months after discharge and evaluated according to the Rankin Revised Scale[6], 63 patients with scores less than 3 (including 3) were in the good prognosis group, and 39 patients with scores greater than 3 were in the poor prognosis group. Logistic regression analysis was used to evaluate the relationship between HA and NLR in peripheral blood and prognosis of patients with sICH.

Statistical methods

IBM SPSS Statistics 25.0 software package was used for statistical data analysis, counting data was expressed as [N (%)], comparison was performed by χ^2 test, and measurement data were compared by means of multiple single-factor samples.

Logistic regression model was used to analyze the relationship between HA, NLR and prognosis of patients with spontaneous intracerebral hemorrhage. ROC curve was used to analyze the prognostic value of peripheral blood HA and NLR alone or in combination. The statistical results were calculated as P< 0.05 was considered statistically significant. Compared with the control group, $^{a}P<0.05$; Compared with good prognosis group, $^{b}P<0.05$.

Results

Comparison of HA and NLR in peripheral blood of patients in each group

The levels of HA and NLR in peripheral blood of the poor/good prognosis group were increased then control group, meanwhile those in the good prognosis group were lower than poor prognosis group (P<0.05). (Table 2).

Group	n	HA (pg/mL)	NLR
Control group	100	212.67±29.31	1.73±0.74
Observation group			
Good prognosis group	63	496.15±32.36[a]	7.92±3.16[a]
Poor prognosis group	39	826.57±28.62[ab]	10.77±3.47[ab]
F		104.39	252.66
P		<0.001	<0.001

Table 2: Comparison of HA and NLR in peripheral blood of each group ($\bar{x}\pm s$).

Comparison of treatment between the two groups

The NIHSS score of good prognosis group was lower than poor prognosis group, and the GCS score was raised (P<0.05). (>Table 3).

Group	n	NIHSS score	GCS score
Good prognosis group	63	6.03±6.53	14.27±1.61
Poor prognosis group	39	21.31±10.07	9.37±3.95
t		9.304	8.761
P		<0.001	<0.001

Table 3: Comparison of treatment between the two groups ($\bar{x}\pm s$).

Hematoma volumes

The average hematoma volume in the good prognosis group (56.79±8.67mL) was smaller than that in the poor prognosis group (91.28±15.96Ml) (P<0.05).

Analysis of independent risk factors for prognosis of patients with acute spontaneous intracerebral hemorrhage

As shown in Table 4 and Figure 1, logistic regression model results showed that NIHSS score, GCS score, HA and NLR were significantly correlated with patient prognosis (P<0.05), were independent risk factors for prognosis of patients with acute sICH.

Related factors	*P*	95% CI	
		Lower limit	Superior limit
Age	0.343	0.992	2.658
NIHSS score	0.024	1.127	2.977
GCS score	0.045	0.098	0.968
HA	<0.001	1.913	6.898
NLR	0.003	0.771	12.573

Table 4: Logistic regression model analysis.

Value of HA and NLR combined detection for prognosis of patients with spontaneous intracerebral hemorrhage

ROC curve results showed that the detection of HA and NLR in peripheral blood alone or in combination has high diagnostic value in analyzing the prognosis of patients with sICH. See Table 5 and Figure 1.

Index	AUC	Sensitivity (%)	Specificity (%)
HA	0.795	68.69	83.46
NLR	0.874	74.22	79.68
HA+NLR	0.915	89.27	94.59

Table 5: Value of HA and NLR combined detection for prognosis of sICH patients.

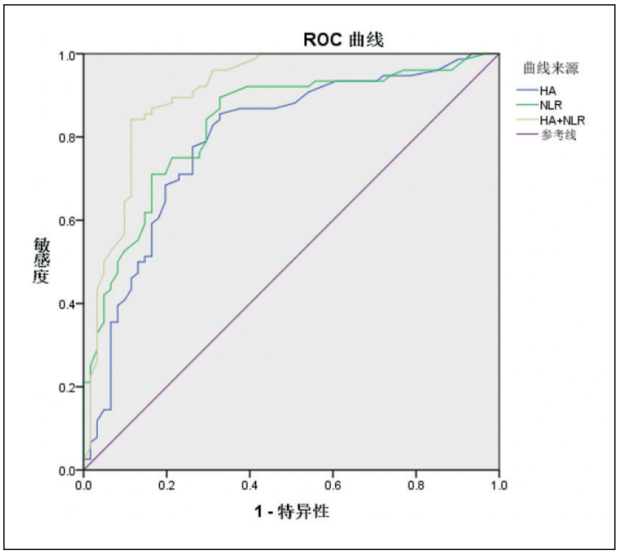

Figure 1: ROC curve of HA and NLR in peripheral blood for prognosis of patients with acute spontaneous intracerebral hemorrhage.

Discussion

sICH, also known as hypertensive encephalopathy, is a disease that has a serious impact on people's life and health, accounting for about 25%~35% of stroke. In Our country, the death rate of cerebrovascular disease is about 3 times that of cardiovascular disease, and now ranks the first in the death rate of Chinese residents.Spontaneous intracerebral hemorrhage is dangerous, recurrent and has poor prognosis[7]. At present, no effective method to reduce the sICH patientsmortality and disability. Therefore, it has become the focus of clinical attention to find indicators that can evaluate the severity of patients' disease and judge the prognosis of patients early. Some studies suggest that secondary injury after intracerebral hemorrhage may

have an important relationship with cerebral edema, vasoactive substances, inflammatory reactions, and oxygen free radicals.

When cerebral hemorrhage occurs in patients, astrocytes secrete a variety of cytokines and inflammatory mediators, resulting in endothelial cell necrosis, which leads to the destruction of the blood brain barrier, increased vascular permeability, and the release of extracellular matrix. Thus further aggravating brain injury[8-9]. Extracellular matrix is a large molecule synthesized by animal cells and secreted out of the cell, mainly existing on the cell surface or between cells[10]. HA level is related to the severity of disease, and inflammatory factors are involved in the pathophysiological process of spontaneous brain injury. HA is a kind of polymer composed, which is a necessary component of extracellular matrix and plays a role in providing a scaffold for cell migration and maintaining cell shape. Meanwhile, HA has a special water-retention effect, and increased HA level can further aggravate brain injury[11]. Khan et al. found in the study of mice that after the removal of CD44 gene, the inflammatory response of brain tissue of mice with cerebral hemorrhage was higher than normal mice, and CD44 is the receptor molecule of HA[12], suggesting that HA is involved in the inflammatory response of brain tissue.The results of this study showed that the NIHSS score in the good prognosis group was lower than poor prognosis group, and GCS score was higher than poor prognosis group (P<0.05). The level of HA in healthy subjects was lower than that in patients with sICH, and it in peripheral blood of patients with good prognosis was lower than poor prognosis (P<0.05).HA level is associated with the severity of sICH and is an important indicator to evaluate the prognosis of patients.

NLR is an effective indicator to reflect systemic inflammatory response and an important indicator to indicate the severity of intracerebral hematoma.Sonpavde et al. proposed for the first time that NLR can predict the prognosis of patients with intracerebral hemorrhage[13]. After cerebral hemorrhage, neutrophils release inflammatory factors and increase vascular permeability, resulting in brain edema.Wang et al. proposed that low lymphocyte count is an independent risk factor for sICH prognosis[14]. Lymphocyte inactivation in necrotic area around intracerebral hemorrhage, reduced lymphocyte count, reduced lymphocyte immune function, resulting in significantly reduced defense ability of nerve cells against inflammatory response.The volume of hematoma in the group with good prognosis was lower than poor prognosis group (P<0.05).The level of NLR in healthy subjects was higher than that in sICHpatients, and the level of NLR in peripheral blood of patients with good prognosis was higher than poor prognosis (P<0.05).

This is consistent with the research results of Alavi et al.[15]. NIHSS score and GCS score can directly reflect the severity of the disease. Brott et al. confirmed that NIHSS score and GCS score scale have good reliability and effectiveness through a number of studies, and they can accurately predict the sICH patient'sprognosis[16]. Logistic regression analysis showed that NIHSS score, GCS score, HA and NLR were independent risk factors for the prognosis of patients with sICH.

In conclusion, peripheral blood HA is a marker of nerve injury with high sensitivity and strong specificity, and NLR is a more economical, effective and widely applied evaluation index. The levels of HA and NLR in peripheral blood can be used as prognostic indicators for patients with spontaneous ich. However, due to the insufficient sample size of this study, the long-term prognostic effect of this study remains to be further analyzed.

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