RESEARCH ARTICLE

The potential role of vascular endothelial growth factor as a new biomarker in severe intracerebral hemorrhage

Jun Zheng¹ | Jianping Sun¹ | Liang Yang¹ | Bin Zhao² | Zhenzeng Fan¹

¹Department of Neurosurgery, The Second Hospital of Hebei Medical University, Shijiazhuang, Hebei, China

²Department of Neurosurgery, The Second Hospital of Jilin University, Changchun, Jilin, China

Correspondence

Zhenzeng Fan, Department of Neurosurgery, The Second Hospital of Hebei Medical University, Shijiazhuang, Hebei, China.
Email: drmakz@163.com

Background: We studied the association between high serum levels of vascular endothelial growth factor (VEGF) and clinical outcomes of intracerebral hemorrhage (ICH) patients.

Methods: Patients were divided into group A (<20 mL), group B (20–30 mL), and group C (>30 mL) based on the bleeding amount. ICH patients were also categorized into the mild group,^{1–15} moderate group (16–30), and severe group (31–45) based on the National Institutes of Health Stroke Scale (NIHSS). The serum levels of VEGF in acute ICH patients detected at 24, 48, and 72 hours were obtained using ELISA kit, and then compared with control group. Main clinical outcomes were evaluated using the modified Rankin scale at 90 days.

Results: The serum levels of VEGF were significantly higher than those in the control group. The serum levels of VEGF in group C were specifically higher compared with those in other two groups. The severe group exhibited higher levels of VEGF than the other two groups. NIHSS scores in patients with good outcomes were lower than those with poor outcomes. Besides, VEGF levels in patients with good outcomes were much higher than those in patients with poor outcomes. ROC results indicated that the optimal cut-off value of VEGF at 72 hours for predicting good outcomes was 111.17 pg/mL with 91.5 sensitivity, 98.7 specificity, and an AUC of 0.952. Our results showed that higher serum levels of VEGF were associated with process of ICH.

Conclusion: VEGF could be a new marker in ICH for severity.

KEYWORDS

acute intracerebral hemorrhage, disease severity, dynamic changes, vascular endothelial growth factor

1 | INTRODUCTION

As suggested by a recent study, the proportion of stroke resulted from intracerebral hemorrhage (ICH) is approximately 20%–30% in Asian regions and this figure is much higher than that in Western countries (10%–15%).¹ Although some clinical treatments have been developed, patients with ICH still exhibit poor clinical outcomes, which results in significantly high mortalities.² Two pathophysiologic courses that are associated with the development of ICH have been revealed³: (i) the expansion of hematoma during the phase of acute development; (ii) the propagation of perihematomal

edema, direct cytotoxicity, the breakdown of blood-brain barrier (BBB), up-regulation of inflammatory factors,⁴ systemic depressed cortical excitability, and apoptosis during the secondary developmental phase.

Over 20 blood biomarkers have been reported to be involved in the prognosis and acute management of ICH such as TNF α , IL-11, and HSP70 etc. As a key vascular permeability factor, vascular endothelial growth factor (VEGF) plays an essential role in the process of physiopathological neovascularization and oxygen delivery.⁵ VEGF is believed to facilitate oxygen delivery by promoting angiogenesis which may influence endothelial cell migration, proliferation,

differentiation, and proteolysis of extracellular matrix. VEGF is believed to perform protective roles in many diseases that are associated with the central nervous system as it was reported to have influence on neurons and glial cells.⁶ Also, it has been reported that serum VEGF levels in acute stroke patients were associated with the improvement in National Institutes of Health Stroke Scale (NIHSS) scores 3 months after admission.⁷ As suggested by mice ICH model experiments, overexpression of VEGF in human neural stem cells promoted neuroprotection, angiogenesis, and functional ischemic recovery.⁸ Besides, overexpression of VEGF in brain tumor cells exacerbates tumor growth and metastasis by promoting neovascularization and increasing microvascular density.⁵ Recent studies have discovered that VEGF expression was closely related to hemorrhage propensity in various tumors. Hemorrhagic transformation usually takes place within 2-3 days after ischemic strokes, and brain ischemia induces the up-regulation of VEGF expression, which reveals the relationship between hemorrhagic transformation and VEGF expression.⁹ On top of that, previous studies have suggested that ICH is able to induce both up-regulation of VEGF and cerebral angiogenesis in animal models.¹⁰ Therefore, regulation of angiogenesis by altering the expression of VEGF may be a potential cure for improving brain damage of ICH.

The induction of abnormal angiogenesis of VEGF in blood vessels has been observed in atherosclerosis and arterial diseases.¹¹ VEGF stimulates angiogenesis by binding to its receptor, VEGF receptor-2, which is a tyrosine kinase and mediates angiogenesis.¹² It is essential to endothelial cell survival and integrity.¹³ However, the underlying relationship between VEGF and strokes, particularly ischemic and hemorrhagic strokes, has not been studied. It has been indicated that VEGF increased the permeability of blood-brain barrier (BBB) which aggravated brain edema.¹⁴ Conversely, VEGF was revealed to alleviate brain edema.¹⁵ Based on what was previously found, the role of VEGF in brain edema, more extensively, in hemorrhagic strokes, needs to be further investigated.

Although brain edema caused by ICH is more severe than cerebral ischemia and it is usually associated with poor prognostic results, few researches have been conducted on how VEGF influences brain edema resulted from ICH. Additionally, no studies have investigated the difference of serum VEGF levels between that at admission and that at hospital discharge. Therefore, this study was specifically designed to assess the association between serum VEGF levels and clinical outcomes of ICH patients.

2 | SAMPLES AND METHODS

2.1 | Study object

Patients who were enrolled within 12 hours post onset of primary ICH symptoms in the Neurology Department of The Second Hospital of Hebei Medical University between January 2012 and December 2013 were included. Inclusion criteria were set as follows: patients who were in line with the diagnostic criteria of hypertension, with cerebral hemorrhage in 20-80 mL and the bleeding site was the basal

ganglia, thalamus and outside the bladder area confirmed by CT. The exclusion criteria were set as follows: patients with secondary ICH resulted from vascular malformation, aneurysm, tumor, hemorrhagic infarction or patients with impaired coagulation, craniocerebral injury, acute diabetic, immunosuppressive treatment, hemorrhagic infarct and patients with a surgical, contraindication of CT or rejection from participation. This exclusion criterion was to ensure that primary ICH patients were strictly distinguished from secondary ICH patients. We are particularly interested in the role of VEGF in primary ICH rather than secondary ICH. A total of 82 patients were recruited including 56 males and 26 females with an average age of 61.35 ± 4.70 (range: 41~80). Clinical information of patients including age, sex, tobacco consumption status and alcohol consumption status, diastolic and systolic blood pressure and results from laboratory tests were collected at admission. Results from head CT scan, which were performed within 24 hours post-ictus,¹⁶ enabled us to divide patients into group A of 38 cases (<20 mL), group B of 25 cases (20-30 mL), and group C of 19 cases (>30 mL) and this classification criterion was based on the bleeding amount calculated using the formula ($\pi/6 \times a \times b \times c$, a=the length-diameter of the largest hematoma plane, b=the diameter of the major perpendicular axis, c=the number of hematoma level). Besides, patients were categorized into the mild group of 24 cases,¹⁻¹⁵ moderate group of 26 cases (16-30) and severe group of 32 cases (31-45) based on the National Institutes of Health Stroke Scale (NIHSS)¹⁷ within 24 hours since admission. Good clinical outcomes were defined for those patients who had modified Rankin Scale (mRs) scores ≤ 2 at 90-day follow-up. A total of 78 healthy subjects who participated in health examinations in the same hospital during the same period were recruited as the control group (average age= 60.15 ± 1.3 , range: 45-78). The implementation of this study complied with the guidelines and regulations set by The Second Hospital of Hebei Medical University. All participants have signed the informed consent and this study was approved by the ethics committee of the Second Hospital of Hebei Medical University.

2.2 | Blood samples and ELISA method

Fasting antecubital vein blood samples of 4 ml were collected from all patients at 24, 48, and 72 hours after the onset of ICH, respectively, blood samples were stored in regular chemistry tubes at room temperature for 0.5 hour. Then, blood samples were centrifuged at 3000 r/m for 10 minutes to separate the serum. Thereafter, the samples were immediately stored in 0.25 mL Eppendorf tubes at -20°C . Another set of 3 mL fasting antecubital vein blood samples were obtained from healthy individuals who were undergoing health examinations once in the morning and sera were isolated and stored under the same procedures as previously described. Serum levels of VEGF in participants were measured with ELISA kits purchased from Jingmei Company (Shenzhen, China), and the instruction was strictly followed. The measurement of VEGF levels was done in an independent laboratory without any background of clinic or neuroimaging.

2.3 | Statistical analysis

Statistical analyses were performed using the SPSS 21 software (IBM). Enumeration or categorical variables are expressed as numbers and percentages. Measurement or continuous data are presented as median values or mean $\pm$ standard deviation (SD). Group differences in categorical variables were analyzed by the χ^2 test, whereas the Mann-Whitney test was used to compare the difference in measurement variables between two groups, and or the Kruskal-Wallis test was used to compare the difference in measurement variables among three groups or more. Spearman analysis was used to verify the correlation between VEGF levels and outcome data (mRs). The receiver operating characteristic curve was drawn to analyze the diagnostic precision. Statistically significant difference was considered when *P* value was below .05.

3 | RESULTS

3.1 | Association between changes in VEGF and ICH

Table 1 exhibits the dynamic changes of serum VEGF levels of ICH patients. Compared with the control group, the serum VEGF levels in ICH patients were significantly higher at 24, 48, and 72 hours ($P < .001$). Meanwhile, the serum levels of VEGF in ICH patients significantly increased 24, 48, and 72 hours after admissions and the serum levels of VEGF at 72 hours were significantly higher than those at 24 hours as seen in Figure 1A.

The association between changes in serum VEGF levels and the bleeding amount was also exhibited in Table 2. The patients were divided into A, B, or C group according to their bleeding amount as previously described. There were significant differences among three groups (all $P < .01$). As the results suggested, the serum levels of VEGF in group C were significantly higher than those in group B and group A at 24, 48, and 72 hours ($P < .001$). Although VEGF level in group B was not statistically different from that in group A at 24 hours, the VEGF level was statistically different from that in group A at 48 and 72 hours, as seen in Figure 1B.

Additionally, we analyzed the relationship between dynamic changes in VEGF levels and ICH severity (Table 3, Figure 1C). ICH patients in the severe group exhibited substantially higher serum levels of VEGF than the mild and moderate groups at 24, 48, and 72 hours

($P < .001$). Similarly, the serum levels of VEGF in the moderate group were significantly higher than the mild group at 24, 48, and 72 hours ($P < .001$). It was evident that high serum levels of VEGF in ICH patients were associated with an increased bleeding amount and severity of ICH, at least 48 hours after admission.

3.2 | Association between changes in VEGF and clinical outcomes

Table 4 provided the main clinical characteristics of patients with good clinical outcomes or poor clinical outcomes. Overall, 47 (57.3%) patients were classified as good clinical outcomes (mRs ≤ 2), whereas 35 (42.7%) were classified as poor clinical outcomes (mRs ≥ 3) during the 3-month follow-up period. Furthermore, patients with good clinical outcomes exhibited significantly lower NIHSS scores on admission compared with ICH patients with poor clinical outcomes ($P < .001$). By contrast, there was no significant difference in age, sex, history of hypertension, history of diabetes mellitus, history of stroke, tobacco consumption status, alcohol consumption status, systolic blood pressure, diastolic blood pressure, leukocyte and platelet between ICH patients with good clinical outcomes, and those with poor clinical outcomes ($P > .05$).

In addition, we measured the VEGF levels of patients in three groups (mild, moderate, severe) and analyzed the difference between patients with good outcomes and those with poor outcomes at 72 hours after admission. No significant difference in VEGF levels was identified between patients with good outcomes and those with poor outcomes in all of the three groups (mild, moderate, severe) (Figure 2A).

Kruskal-Wallis test were conducted to determine whether the serum VEGF levels were associated with the clinical outcomes of ICH patients. The results showed in Table 5 and Figure 2B suggested that the serum levels of VEGF in patients with good clinical outcomes significantly exceeded those with poor clinical outcomes when they were measured in 24, 48, and 72 hours after admission ($P = .039$, $P < .001$, $P < .001$, respectively).

The evidence above implied that higher serum levels of VEGF were associated with more optimistic or desirable clinical outcomes of ICH patients. We also used ROC analysis to determine the precision of serum levels of VEGF in predicting ICH patients who were likely to exhibit desirable clinical outcomes. As Figure 2C showed, VEGF levels may provide reliable diagnostic information for differentiating ICH patients with good clinical outcomes from those with poor clinical outcomes with an area under the curve (AUC) of 0.952. Additionally, the ROC curve revealed that the optimal cut-off value of VEGF serum level detected within 72 hours on admission for distinguishing between ICH patients with favorable outcomes and with poor outcomes was 111.17 pg/mL. The specificity and sensitivity were 91.5% and 98.7%, respectively.

TABLE 1 The VEGF level in serum of ICH patients in different time

Time point (hours)	VEGF (pg/mL)		P value
	Control	ICH	
24	83.66 $\pm$ 15.45	149.03 $\pm$ 44.97	<.001
48		161.72 $\pm$ 41.58	<.001
72		173.61 $\pm$ 49.48	<.001

Mann-Whitney test was used to compare the level of VEGF in control and case group in 24, 48 and 72 hours.

4 | DISCUSSION

The main results from this study on ICH patients were summarized as: (i) serum VEGF level was positively correlated with hemorrhage

FIGURE 1 A, Serum VEGF levels in ICH patients and non-ICH patients at 24, 48, and 72 hours after admission, respectively. *** means there was significantly different between 24 and 72 hours, $P < .001$. B, Serum VEGF levels in ICH patients in group A, B, and C (groups were divided based on head CT results and patients in different groups had different hemorrhage volumes) at 24, 48, and 72 hours after admission, respectively. * means there was significantly different between group A and C, $P < .05$; & means there was significantly different between group B and C, $P < .05$; # means there was significantly different between group A and B, $P < .05$. C, Serum VEGF levels in ICH patients in mild, moderate and severe groups (groups were divided based on NIHSS scoring system) at 24, 48, and 72 hours after admission, respectively. * means there was significantly different between group A and C, $P < .05$; # means there was significantly different between group A and B, $P < .05$

Time point (hours)	VEGF(pg/mL)			P value
	A	B	C	
24	118.91±26.53	134.06±17.49	184.44±24.32	<.001
48	130.99±29.85	151.81±14.17	214.31±23.63	<.001
72	142.24±31.04	162.71±13.73	228.26±23.52	<.001

Kruskal-Wallis test was used to compare the VEGF level in different bleeding group in different time.

Time point (hours)	VEGF (pg/mL)			P value
	Mild	Moderate	Severe	
24	111.58±24.43	138.27±25.40	174.27±25.74	.001
48	125.09±23.62	156.73±23.39	202.72±30.32	<.001
72	134.4±22.65	168.79±25.14	211.26±30.59	<.001

Kruskal-Wallis test was used to compare the level of VEGF level among mild, moderate, and severe group.

TABLE 2 The association of VEGF level with the bleeding amount

TABLE 3 The association of VEGF level with the disease severity

volume and severity of ICH; (ii) ICH patients with higher VEGF serum levels exhibited more optimistic clinical outcomes over the 3-month following up period. We thus suspected that ICH patients with higher serum levels of VEGF are associated with an increased chance of recovery from ICH.

In this study, dynamic changes in serum levels of VEGF in 82 ICH patients and the main clinical outcomes of these patients assessed by mRs over a period of 90 days were both investigated. Our study verified that serum levels of VEGF in ICH patients were significantly higher than those in healthy individuals and they increased as the detection time extended. Furthermore, ICH patients with larger volume of hemorrhage in our study exhibited higher serum levels of VEGF. As suggested by the NIHSS, severe ICH patients also exhibited higher serum levels of VEGF compared to the moderate and mild group. These conclusions may seem contradict since a more severe ICH usually results in a more undesirable prognosis, however, the rising trend of VEGF in severe patients might be a result of cell death and thus generated VEGF was induced into the blood. On the other hand, the association of the increase of VEGF expression after the brain injury with a better prognosis may lie in the angiogenesis

process which would promote recovery. Thus, significant correlations between ICH severity/hemorrhage volume and VEGF serum concentration suggested that VEGF might be involved in the following repair processes when stroke occurs and the result was similar to that of researches conducted on animal models.¹⁰ VEGF is believed to be an endothelial cell-specific factor which plays a fundamental role in the course of blood vessel formation. In addition, VEGF has been validated to play a protective role in numerous neurological diseases as it protects glial cells and neurons from attacks.⁶ Plenty of studies conducted on cerebral ischemic models have demonstrated the protective effects of VEGF on neurons via, for instance, inhibiting apoptosis and reducing the oxidative stress in acute cerebral ischemic, and it is able to promote neurogenesis and enhance angiogenesis in chronic cerebral ischemic.¹⁸

ICH is considered to be a fatal type of stroke with approximately 50% mortality,¹⁹ however, the therapeutic approaches mainly focus on alleviating hematoma with very limited effectiveness,⁸ thus improving the prognostic outcomes of patients with ICH remains challenging.²⁰ Plethora of blood biomarkers have been used in the management of intracerebral hemorrhage such as S100 beta.²¹

TABLE 4 Baseline clinical and laboratory characteristics in patients with good or poor outcome

Clinical/Laboratory data	Good outcome (n=47)	Poor outcome (n=35)	P value
Age	60.5±11.7	64.6±11.5	.096
Gender (male/female)	33/14	23/12	.665
History of hypertension	39	28	.730
History of diabetes mellitus	10	9	.638
History of stroke	6	2	.287
Tobacco use	13	12	.519
Alcohol use	17	12	.860
NIHSS	6.5±1.5 (4-11.5)	14.2±2.3 (11-16.5)	<.001
Systolic blood pressure	172.35±23.61	181.13±27.35	.266
Diastolic blood pressure	97.15±13.95	97.26±17.15	.871
VEGF levels at 72 hours			
Mild (n)	14	10	.987
Moderate (n)	15	11	
Severe (n)	18	14	
Leukocyte (×10 ³ /mL)	8.9±2.3	9.5±3.2	.204
Platelet (×10 ³ /mL)	235.6±54.5	228.6±44.9	.618

Data are expressed as n or mean±SD as appropriate. Chi-square test was used for enumeration variables; Mann-Whitney test was used for continuous variables.

FIGURE 2 A, Serum VEGF levels at 72 hours after admission in ICH patients with good or poor outcomes in mild, moderate, and severe groups (groups were divided based on NIHSS scoring system). B, Serum VEGF levels at 24, 48, and 72 hours after admission in patients with good or poor outcomes. *means there was significantly different between good outcome and poor outcome, $P<.05$; ***means there was significantly different between good outcome and poor outcome, $P<.001$; **** means there was significantly different between good outcome and poor outcome, $P<.0001$. C, ROC curve of serum VEGF levels in ICH patients**TABLE 5** VEGF levels at different time point by functional outcome

Time point (hours)	VEGF (pg/mL)		P value
	Good outcome (n=47)	Poor outcome (n=35)	
24	159.63±42.81	134.79±44.45	.039
48	174.55±43.71	144.46±31.58	<.001
72	193.42±47.72	147.00±38.47	<.001

Mann-Whitney test was used to compare the VEGF level in good outcome and poor outcome group at different time.

Previous studies have suggested that VEGF was correlated with the improvement in NIHSS scores in acute ischemic stroke patients treated with it for 3 months.⁷ Meanwhile, we discovered that VEGF

serum levels in ICH patients with good clinical outcomes were much higher than those in ICH patients with poor outcomes over a 3-month following up period. Further evidence suggested that VEGF could reduce neuronal death. Recently, an experiment conducted on mice models verified the positive effects of VEGF on neuronal death elimination, neurological defects reduction, and brain edema relief triggered by the up-regulation of the aquaporin-4 (AQP4) which had a serious impact on decreasing brain edema that resulted from ICH.⁵ It was likely that VEGF could down-regulate the neurotoxic proteins, whereas it is able to up-regulate neurotrophic and anti-apoptotic proteins such as the pigment epithelial-derived factor (PEDF) in order to protect the neural cells from apoptosis.²² This protection may be contributed by the influence of VEGF on the inhibitor κ B-a (κ B-a) phosphorylation and the nuclear translocation of the transcription factor κ B (NF κ B).²³ It is also suggested that VEGF could

induce recovery from ICH through mobilizing the connection between macrophage inflammation and angiogenesis. Previous studies have discovered an increased microvessel density triggered by VEGF in those regions involving macrophages.²⁴ Therefore, VEGF may provide more inflammatory responses and facilitate the clearance of hematoma.²⁵

On top of that, our study may provide additional insights into tailoring ICH therapies for patients with different clinical outcomes. As suggested by the ROC analysis, the optimal cut-off value of VEGF detected at 72 hours for predicting clinical outcomes of ICH patients was 161.68 pg/mL with an AUC of 0.608, which implied a relatively precise diagnostic method. Sobrino et al. revealed that serum VEGF ≥ 330 pg/mL detected at 72 hours was independently associated with good clinical outcomes and decreased lesion volumes.²⁵ Another study on intraventricular hemorrhage (IVH) among preterm neonates indicated that the appropriate cut-off value of VEGF was 135 pg/mL with a 96% sensitivity and 100% specificity.²⁶ Though the optimal cut-off value of VEGF for determining clinical outcomes of patients varied across different studies, the significant effects of VEGF on predicting hemorrhage status have been demonstrated by a number of VEGF-related studies.

One limitation of our study was the relatively short follow-up period which did not allow us to determine whether these desirable clinical outcomes were triggered by transient increase in the serum VEGF level or a continuous up-regulation of VEGF during the follow-up period. Therefore, further studies are needed to be conducted to address this issue and ascertain the conclusions above. Another limitation would be the mean time to CT from onset of symptoms. According to Fujii et al.,²⁷ hematoma volume increased significantly within the first 2 or 3 hours after ICH onset and the change rate started to decrease 6 hours after onset. The stabilization of the hematoma volume should be used for correlation analysis. However, the hematoma volume is associated with many other factors such as the neurologic deterioration, thus the unstable condition of the hematoma volume expansion should be seriously taken into consideration.

In summary, we asserted that the elevated serum levels of VEGF were associated with pathogenesis of ICH and because of which, VEGF could be a new marker in ICH for severity.

ACKNOWLEDGEMENTS

We thank Prof. Gengsheng Zhang (The Second Hospital of Hebei Medical University) for kindly providing the academic help.

REFERENCES

- van Asch CJ, Luitse MJ, Rinkel GJ, van der Tweel I, Algra A, Klijn CJ. Incidence, case fatality, and functional outcome of intracerebral haemorrhage over time, according to age, sex, and ethnic origin: A systematic review and meta-analysis. *Lancet Neurol*. 2010;9:167–176.
- Qureshi AI, Mendelow AD, Hanley DF. Intracerebral haemorrhage. *Lancet*. 2009;373:1632–1644.
- Chang JJ, Emanuel BA, Mack WJ, Tsvigoulis G, Alexandrov AV. Matrix metalloproteinase-9: Dual role and temporal profile in intracerebral hemorrhage. *J Stroke Cerebrovasc Dis*. 2014;23:2498–2505.
- Belur PK, Chang JJ, He S, Emanuel BA, Mack WJ. Emerging experimental therapies for intracerebral hemorrhage: Targeting mechanisms of secondary brain injury. *Neurosurg Focus*. 2013;34:E9.
- Chu H, Tang Y, Dong Q. Protection of vascular endothelial growth factor to brain edema following intracerebral hemorrhage and its involved mechanisms: Effect of aquaporin-4. *PLoS ONE*. 2013;8:e66051.
- Ruiz de Almodovar C, Lambrechts D, Mazzone M, Carmeliet P. Role and therapeutic potential of VEGF in the nervous system. *Physiol Rev*. 2009;89:607–648.
- Lee SC, Lee KY, Kim YJ, Kim SH, Koh SH, Lee YJ. Serum VEGF levels in acute ischaemic strokes are correlated with long-term prognosis. *Eur J Neurol*. 2010;17:45–51.
- Lee HJ, Kim KS, Park IH, Kim SU. Human neural stem cells over-expressing VEGF provide neuroprotection, angiogenesis and functional recovery in mouse stroke model. *PLoS ONE*. 2007;2:e156.
- Kovacs Z, Ikezaki K, Samoto K, Inamura T, Fukui M. VEGF and flt. Expression time kinetics in rat brain infarct. *Stroke*. 1996;27:1865–1872; discussion 1872–1863.
- Tang T, Liu XJ, Zhang ZQ, et al. Cerebral angiogenesis after collagenase-induced intracerebral hemorrhage in rats. *Brain Res*. 2007;1175:134–142.
- Khurana R, Simons M, Martin JF, Zachary IC. Role of angiogenesis in cardiovascular disease: A critical appraisal. *Circulation*. 2005;112:1813–1824.
- Cui HJ, Yang AL, Zhou HJ, et al. Buyang huanwu decoction promotes angiogenesis via vascular endothelial growth factor receptor-2 activation through the PI3K/Akt pathway in a mouse model of intracerebral hemorrhage. *BMC Complement Altern Med*. 2015;15:91.
- Domigan CK, Warren CM, Antanesian V, et al. Autocrine VEGF maintains endothelial survival through regulation of metabolism and autophagy. *J Cell Sci*. 2015;128:2236–2248.
- Bates DO. Vascular endothelial growth factors and vascular permeability. *Cardiovasc Res*. 2010;87:262–271.
- Miki Y, Nonoguchi N, Ikeda N, Coffin RS, Kuroiwa T, Miyatake S. Vascular endothelial growth factor gene-transferred bone marrow stromal cells engineered with a herpes simplex virus type 1 vector can improve neurological deficits and reduce infarction volume in rat brain ischemia. *Neurosurgery*. 2007;61:586–594; discussion 594–585.
- Zimmerman RD, Maldjian JA, Brun NC, Horvath B, Skolnick BE. Radiologic estimation of hematoma volume in intracerebral hemorrhage trial by CT scan. *AJNR Am J Neuroradiol*. 2006;27:666–670.
- Qiu SZ, Wang HX, Shen J, et al. The prognostic value of serum signal peptide-Cub-Egf domain-containing protein-1 concentrations in acute intracerebral hemorrhage. *Clinica Chimica Acta*. 2016;461:103–109.
- Yang JP, Liu HJ, Liu XF. VEGF promotes angiogenesis and functional recovery in stroke rats. *J Invest Surg*. 2010;23:149–155.
- Gebel JM, Broderick JP. Intracerebral hemorrhage. *Neurol Clin*. 2000;18:419–438.
- Lee CZ, Xue Z, Zhu Y, Yang GY, Young WL. Matrix metalloproteinase-9 inhibition attenuates vascular endothelial growth factor-induced intracerebral hemorrhage. *Stroke*. 2007;38:2563–2568.
- Senn R, Elkind MS, Montaner J, Christ-Crain M, Katan M. Potential role of blood biomarkers in the management of nontraumatic intracerebral hemorrhage. *Cerebrovasc Dis*. 2014;38:395–409.
- Sanchez A, Wadhvani S, Grammas P. Multiple neurotrophic effects of VEGF on cultured neurons. *Neuropeptides*. 2010;44:323–331.
- Zachary I. Neuroprotective role of vascular endothelial growth factor: Signalling mechanisms, biological function, and therapeutic potential. *Neurosignals*. 2005;14:207–221.
- Manonkitiwongsa PS, Schultz RL, Whitter EF, Lyden PD. Contraindications of VEGF-based therapeutic angiogenesis: Effects

- on macrophage density and histology of normal and ischemic brains. *Vascul Pharmacol*. 2006;44:316–325.
25. Sobrino T, Arias S, Rodriguez-Gonzalez R, et al. High serum levels of growth factors are associated with good outcome in intracerebral hemorrhage. *J Cereb Blood Flow Metab*. 2009;29:1968–1974.
26. El Shimi MS, Hassanein SM, Mohamed MH, et al. Predictive value of vascular endothelial growth factor in preterm neonates with intraventricular haemorrhage. *J Matern Fetal Neonatal Med*. 2012;25:1586–1590.
27. Fujii Y, Tanaka R, Takeuchi S, Koike T, Minakawa T. Hematoma enlargement in spontaneous intracerebral hemorrhage. *J Neurosurg*. 1994;80:51–57.