Original Article
Joint detection of serum Cys-C, IL-6 and VEGF levels in patients with hypertensive acute cerebral infarction

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Abstract: Monitoring serum levels of key factors involved in acute cerebral infarction (ACI) was recently accepted as a novel prognostic strategy. However, by now there were still no practical serum indicators for ACI with any other symptoms. Our work aims to figure out the prognostic biomarkers for ACI with hypertension. We recruited 148 hypertensive ACI patients (HACI), 50 ACI only patients and 50 healthy volunteers for this study. The HACI patients were further divided into 3 subgroups with different severity, 36 sever, 50 medium, and 62 mild according to the infarction size measured by MRI which were also confirmed by NIHSS score. To monitor this complicated brain cardiovascular problem, we detected the serum levels of three factors involving in independent stroke events, Cys-C, VEGF and IL-6 at day 1, day 3, day 7 and day 14 after the recruitment respectively. At day 1, the serum levels of three factors in HACI patients are all higher than ACI only and healthy groups. For the following days, the serum levels of three factors in all the HACI subgroups were still higher than healthy group. Further, the serum levels of these three factors were all positively correlated with the severity in HACI patients. Each factors had a unique curve for the 14 days detection and therefore joint detection at different time point could provide more information for HACI. Collectively, our work suggested Cys-C, VEGF and IL-6 could either worked as independent prognostic indicators or worked together for better evaluation of HACI.

Keywords: Cys-C, IL-6, VEGF, acute cerebral infarction, hypertension, joint detection

Introduction

Acute cerebral infarction (ACI) is one of the most serious brain cardiovascular diseases in the modern society, resulting in great burden for both the families and the whole society [1]. Hypertension or high blood pressure is also a cardiovascular problem affecting almost one out of seven people worldwide [2-4]. These two diseases were highly related with each other and cross-talked frequently. On one hand, hypertension was the main risk factor for ACI besides age, [5] although the mechanisms of hypertension causing stroke remain unclear because of the complexity of stroke [6]. On the other hand, ACI could also cause temporary hypertension especially in the early period of both ischemic and hemorrhagic stroke [7, 8]. The interactions between two diseases and the unclear mechanism made hypertensive ACI more challenging for prognosis and investigation [8].

Studies have suggested that serum levels of some independent key factors involve in ischemic stroke could be the prognostic indicators for infarct size in ACI only patients, such as Cystain C (Cys-C), Interleukin 6 (IL-6) and vascular endothelial growth factor (VEGF) [9-11]. Cys-C is characterized as the inhibitor of cysteine proteases and its penetration through the glomerular filtration membrane could be regarded as a sensitive renal function biomarker [12]. Furthermore, recent studies found that serum Cys-C level was also a strong predictor of the risk of cardiovascular events which could affect the infarct size, or hemorrhage volume and indicate the severity of cerebral microbleeds in acute cerebral stroke events [9, 13, 14]. IL-6 was one of the key cytokines reported in the inflammatory events after stroke and the level in cerebrospinal fluid raised significantly after infarction, which suggested that IL-6 could also serve as a reliable prognostic factor for ischemic stroke [10, 15]. VEGF is a well-known

Table 1. Characteristics of the patients

Groups	Case Number	Gender (Male/Female)	Age (Years)	BMI (Kg/m^2)	Nicotine abuse (n/ratio)	Alcohol abuse (n/ratio)
HACI	148	95/53	65.9±6.3	24.32±1.81	12 (8.1%)	8(5.4%)
ACI	50	31/19	63.1±7.8	24.54±2.01	3 (6.0%)	2(4.0%)
Control	50	29/21	64.2±5.6	23.61±1.96	4 (8.0%)	3 (6.0%)
P value*		0.603	0.899	0.985	0.231	0.423
P value**		0.639	0.856	0.684	0.643	0.763
P value***		0.732	0.779	0.691	0.563	0.456

Note: *: Comparison between HACI and ACI only group. **: Comparison between HACI and control group. ***: Comparison between ACI only and control group.

Table 2. Hypertensive ACI subgrouping

subgroups/Volume	case number	Gender (Male/Female)	Age (year)	BMI (kg/m^2)	infarct size (diameter/cm)	NIHSS
large	36	23/13	64.9±4.8	24.21±2.34	7.23±0.81	27.25±2.80
medium	50	32/18	66.3±5.4	24.48±1.36	3.94±0.42	14.75±1.63
small	62	40/22	65.6±3.2	24.29±2.54	2.15±0.38	3.25±0.75
P value*		0,621	0.435	0.672	0.03	0.001
P value**		0.424	0.532	0.535	0.04	0.02
P value***		0.654	0.312	0.331	0.004	0.003

Note: *: Comparison between large and medium groups. **: Comparison between medium and small groups. ***: Comparison between large and small groups.

molecule mediating neuronal survival and angiogenesis in the physiological state and pathological states, including ischemic infarction [11, 16]. The expression level of VEGF increased after acute infarction [17] and VEGF was also reported as prognostic indicator for long term effect after stroke.

To establish a prognosis biomarker for hypertensive acute cerebral infarction (HACI), we detected the serum levels of Cys-C, IL-6, and VEGF in HACI patients with different severity and at different time points. We found these factors could all work as indicators of HACI. Our work also suggested that the joint detection of these three different factors at different time point could help predict three different aspects of stroke events.

Materials and methods

All the people involved in this research were ACI patients or healthy volunteers from our hospital between September 2011 and September 2013. The inclusive criteria for ACI were laid out as following: 1. All the ACI patients were first-ever stroke onset and were in accordance with

the inclusion criteria proved by the Chinese Neuroscience Society; 2. All the patients were confirmed by MRI scanning; 3. The duration should be less than 72 hours. The inclusion criteria for hypertension were in accordance with Guidelines for Prevention and Treatment of Hypertension in China (2005 version). The exclusion criteria were patients with the following characteristics: 1. hemorrhagic stroke; 2. duration more than 3 days; 3. acute and chronic infections, kidney disease, connective tissue disease, cancer, diabetes, coronary heart disease, anemia and other vital organs disease history; 4. receiving surgery within three months; 5. relevant laboratory examinations show the indicators were not within the normal range. ACI patients recruited would receive conventional treatment, including anticoagulation, antiplatelet, reducing lipid levels, improving microcirculation, brain cell activator treatment and so on.

Blood sample collection

Blood was collected from each group of patients with at least 8 hrs fasting at day 1, day 3, day 7 and day 14 respectively. Serum was isolated
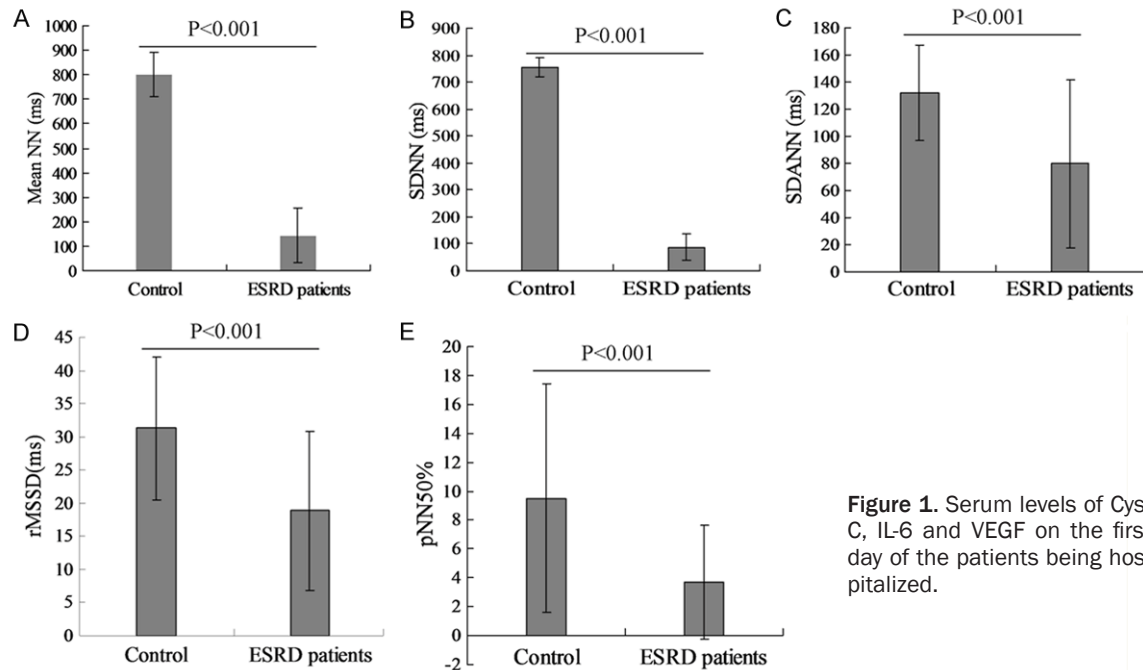
Figure 1. Serum levels of Cys-C, IL-6 and VEGF on the first day of the patients being hospitalized.

and stored at -20°C with 0.25 ml aliquot in each Eppendorf tube to avoid repeated freezing and thawing.

Serum levels detection for Cys-C, IL-6 and VEGF

Serum Cys-C was determined by particle-enhanced immunoturbidimetric method (Cys-C kit: Beijing Leadman Companies), and the instrument was the Hitachi 7600 automatic biochemical analyzer Miriam; serum levels of IL-6 and VEGF were determined by double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) (VEGF kit: Accessories Maixin biotechnology Co., Ltd. to provide; IL-6 kit: on Haixi Tang biological Technology Co., Ltd.), and the equipment was the US BEP2000 automatic microplate reader. We followed strictly the kit instructions to detect the levels for Cys-C, IL-6 and VEGF.

Statistical analysis

SPSS17.0 statistical software was used to analyze data; the data are shown as X ± SD. One-way ANOVA was used to do further pair wise comparisons and LSD-t test, Spearman rank correlation analysis were used for correlation analysis. P<0.05 indicates that there was statistically significant difference.

Results

Information for the patients in the three groups

Characteristics of all the ACI patients and healthy controls were listed in **Table 1**. Among the 148 hypertensive ACI patients, there were 95 male and 53 female, with the average age of 65.9 and normal body mass indexes (24.3). The ratio of nicotine abuse and alcohol abuse were both at low rate (less than 10%) (**Table 1**). We also recruited 50 ACI only patients and 50 healthy volunteers as two control groups, which did not show any statistical differences in terms of gender ratio, average age, average BMI, nicotine abuse ratio and alcohol abuse ratio (**Table 1**). HACI group was further divided into 3 subgroups according to the infarct size: 36 cases large size group (diameter >5 cm), 50 cases medium size group (diameter >3 cm, <5 cm), and 62 cases small size group (diameter >1.6 cm, <3 cm) (**Table 2**). The infarct size was also confirmed by neurological evaluation method, the National Institutes of Health Stroke Scale (NIHSS) score to assess the patient's state of consciousness and neurological conditions [18] (**Table 2**). The results shown that as the infarct size increased, the NIHSS increased.
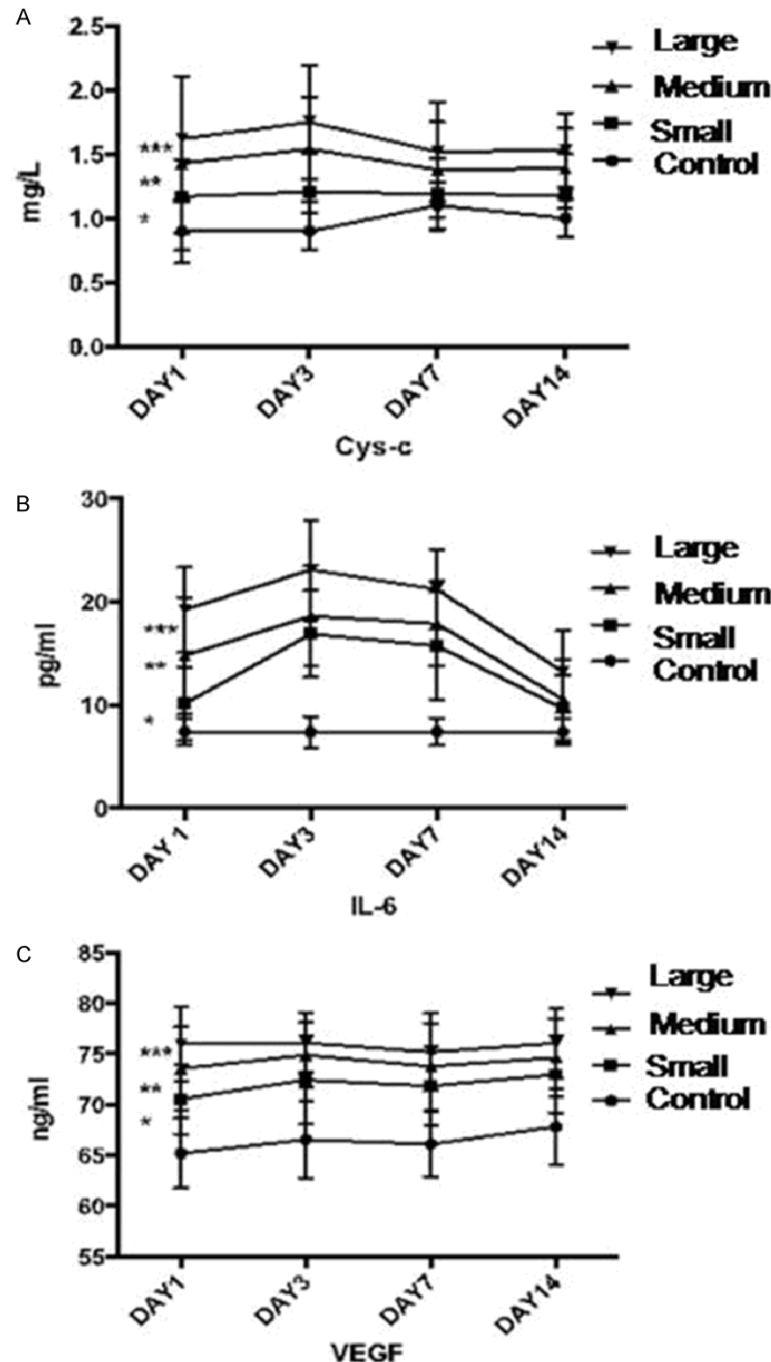
Figure 2. Serum levels of Cys-C, IL-6 and VEGF at different days after the patients with different infarction size being hospitalized.

Comparisons of Cys-C, IL-6 and VEGF levels in serum

On the first day of patients being hospitalized, we detected the levels of Cys-C, IL-6 and VEGF in the serum among different groups and we found that there were statistically significant differences among the three groups: for all of the three factors-Cys-C, IL-6, VEGF, the level for the hypertensive group is the highest, ACI only group lies in the middle, and the control group is the lowest (**Figure 1**).

To see if the levels of the three factors correlate with the infarction size, we also detected the levels of Cys-C, IL-6 and VEGF in the serum among different infarction groups. Results showed that in the HACI group, the levels of Cys-C, IL-6 and VEGF are positively correlated with the ACI infarction size at days 1, 3, 7 and 14 and the differences are statistically significant (**Figure 2**).

Correlation analysis

The levels of Cys-C, IL-6 and VEGF are positively correlated with the ACI infarction volume at days 1, 3, 7 and 14 in the HACI groups. The correlation index is shown in the **Table 3**.

Discussion

Acute cerebral infarction is one of the highly deadly and disabled vascular diseases, and accurately evaluating its severity and prognosis may be of great importance. As more and more researches, the relationship between inflammatory response and ACI has been paid more attention. After infarction, inflammation directly plays a role in brain damage and both inflammatory cells and inflammatory cytokines are involved in the process [19]. Thus, inflammatory cytokines would be very valuable for determining the occurrence of cerebral infarction and its prognosis.

Table 3. Correlation between the levels of Cys-C, IL-6 and VEGF in the serum for the hypertensive ACI group and ACI infarction volume

Detection time	Cys-C (mg/L)	IL-6 (pg/ml)	VEGF (ng/ml)
Day 1	r=0.337, P=0.042	r=0.451, P=0.032	r=0.549, P=0.008
Day 3	r=0.519, P=0.006	r=0.503, P=0.018	r=0.549, P=0.008
Day 7	r=0.329, P=0.038	r=0.325, P=0.037	r=0.549, P=0.008
Day 14	r=0.259, P=0.048	r=0.089, P=0.120	r=0.339, P=0.042

Our studies have found that the IL-6 level in the ACI group is significantly higher than the control group and the difference is statistically significant; the IL-6 level positively correlates with the size of the infarction; the level of IL-6 in the hypertensive ACI group is significantly higher than simple ACI patients. Possible explanation would be that the increase of IL-6 may result in the increasing expression of MMP-1 [20], and MMP-1 would participate in the breakdown of brain blood barrier and induce the exacerbation of brain damage. Other literatures have reported that IL-6 would induce the expression of phospholipase A2, which may degrade the phospholipids component of the cellular membrane and produce those inflammatory cytokines, such as leukotriene and platelet stimulating factor, and gradually induce the transformation of the atherosclerotic plaque, resulting in the increase susceptibility of stroke [15]. Cystatin C has long been regarded as one of the parameters for kidney function detection. Recently, Cys-C has been reported as the independent risk factor for cerebral infarction [21], and the increase has been correlated with the risk for atherosclerosis thus for the development of stroke. VEGF also showed positive correlation with the infarction volume. And this may be due to the evidence that VEGF could exacerbate blood-brain barrier (BBB) disruption after ischemic stroke through Orm1 and its inhibition of the NF-κB pathway [22].

Our studies have found that joint detection of the levels of CysC, IL-6 and VEGF positively correlated with the infarction volume of HACI, and this result is the first report that detecting three risking factors at the same time. Our studies have shed instruction to the further diagnostic biomarkers for stroke treatment.

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Disclosure of conflict of interest

None.

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