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Plasma concentration of ZCCHC14 contributes to prognostic efficacy in intracerebral hemorrhage patients

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Abstract. – OBJECTIVE: Intracerebral hemorrhage (ICH) is a devastating cerebrovascular disease of the central nervous system. In this study, we aimed to investigate plasma ZCCHC14 expression and its correlation with the diagnosis, prognosis, and clinical features of ICH.

PATIENTS AND METHODS: The plasma levels of ZCCHC14, TNF-α, IL-6, and IL-10 were dynamically detected among 130 ICH patients and 60 corresponding healthy subjects. A receiver operating characteristic (ROC) curve was used for the statistical analysis of the diagnostic and prognostic efficacy of ICH.

RESULTS: Hypertension ($p = 0.005$), ARB application ($p = 0.014$), ZCCHC14 level ($p < 0.001$), TNF-α ($p < 0.001$), IL-6 ($p < 0.001$), and IL-10 ($p < 0.001$) were found to be different between ICH patients and healthy controls. Multiple logistic regression analysis indicated that ZCCHC14 levels at admission were significantly different between the two groups ($p = 0.002$, OR = 0.440, 95% CI 0.290-0.718). Plasma ZCCHC14 levels dynamically changed, increasing at admission, and peaking on day 7. ZCCHC14 was a potential diagnostic marker for ICH (AUC = 0.953, $p < 0.0001$, 95% CI: 0.901-1.004) with a specificity and sensitivity of 84.6% and 95.5%, respectively. The plasma ZCCHC14 level was negatively correlated with IL-6 concentration ($p = 0.024$, $r = -0.311$) but was positively correlated with IL-10 concentration ($p = 0.041$, $r = 0.298$). Furthermore, the plasma ZCCHC14 level was correlated with hypertension ($p = 0.005$), GOS ($p = 0.025$), bleeding volume ($p < 0.001$), midline shift ($p = 0.003$), and poor outcome ($p = 0.006$). The low ZCCHC14 expression group had poorer outcomes (death or severe disability) than high ZCCHC14 expression group (Breslow, $p < 0.001$).

CONCLUSIONS: We found that the plasma ZCCHC14 level might be a potential biomarker for both the early diagnosis of and prediction of outcomes in ICH.

Key Words:
Intracerebral hemorrhage, ZCCHC14, Receiver operating characteristic, Diagnosis, Prognosis.

Introduction

Intracerebral hemorrhage (ICH) represents a series of devastating diseases in the central nervous system (CNS) and occurs when diseased brain blood vessels rupture, causes the release of blood into the surrounding brain parenchyma. Although ICH accounts for just 10%-15% of all stroke cases in high-income countries and there is an even higher percentage in Asia, it often leads to a severe prognosis with a one-month mortality rate of approximately 40% in the acute phase of the disease[1]. Since standard management is limited to primarily supportive treatments, such as the reduction of brain oedema, control of intracranial pressure, and maintenance of hemodynamic stability, researchers have found that controlling excessive pro-inflammation and balancing the effects between anti- and pro-inflammation in the stage of secondary brain injury might be effective therapeutic options that may increase the survival rate and improve neurological recovery in ICH patients[2-4].

In clinical practice, imageological techniques such as computed tomography (CT) and CT angiography (CTA) have been widely applied and have excellent diagnostic efficacy in ICH[5]. However, there are still some misdiagnoses based on the use of imageological manifestations alone. Moreover, the prognosis of ICH patients can hardly be predetermined by imageological indications.

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Previous investigations[6,7] have indicated that ICH is a complex disease, of which both genetic backgrounds and environmental factors contribute mutually to the risk. In recent decades, with the development of omics-based high-throughput methods, numerous ICH-related genes have been found to be significantly associated with the risks and outcomes of ICH. Yamada et al[8] found that DNTTIP2 and FAM205A contributed to the susceptibility to ICH in a Japanese cohort. Ilinca et al[9] identified a large gene cohort that was associated with the risk of stroke in a European population: 120 susceptibility genes were associated with stroke, and 62 susceptibility genes were associated with diseases that might cause stroke. Moreover, several other ICH-related genes were also discovered, such as APOE, COL4A1, and COL4A2 genes[10-12]. In a recent genome-wide association study (GWAS), a single nucleotide polymorphism (SNP) rs12445022 was found to be significantly associated with ZCCHC14 mRNA expression and the risk of early onset of small vessel stroke (SVS)[13]. In addition, we did not find any evidence of ZCCHC14 expression associated with diagnostic and prognostic values in ICH.

In this study, we dynamically tested plasma levels of ZCCHC14 and some ICH-related cytokines, aiming at investigating plasma ZCCHC14 expression and its correlation with the diagnosis, prognosis, and clinical features of ICH.

Patients and Methods

Study Population

A hospital-based case-control study was conducted from May 2015 to October 2016. A total of 130 patients with ICH and 60 sex- and age-matched healthy controls were recruited from the First Affiliated Hospital of Anhui Medical University and Lu'an Second People's Hospital. There were no age or sex differences between the two groups. A definite and consistent diagnosis of ICH was made by two senior neurosurgeons. Unrelated, matched healthy donors were recruited from the physical examination centre. Individuals who had cancer, autoimmune diseases, systemic diseases, or other intracranial diseases were excluded from both case group and control group. Informed consent was provided by the patients and control subjects. This investigation was approved by the Clinical Research Ethics Committee of both hospitals and conducted according to the principles Declaration of Helsinki.

Clinical Characteristics and Sample Collection

Demographic data and clinical characteristics were collected for all patients, including age, sex, disease and medication history, ICH volume, midline shift, and intraventricular hemorrhage (IVH). Furthermore, all patients received neurological damage checks, which were assessed with the Glasgow Coma Scale (GCS) at admission. Survival status for all the patients was recorded on day 30 after admission, describing normal status, and severe disability to mortality. Recovery outcomes were assessed using the Glasgow Outcome Scale (GOS) in the 3rd month. The evaluation of scoring was conducted by two experienced senior doctors. Plasma was drawn via forearm venepuncture into evacuated ethylenediaminetetraacetic acid (EDTA)-coated tubes from each patient at different time points on day 1 (on the day of admission and within 4 h of disease onset), day 3, day 7, day 14, and day 30 after admission, and the plasma was placed into evacuated EDTA-coated tubes. All plasma samples were stored at –80°C.

Enzyme-Linked Immunosorbent Assay (ELISA)

All ELISA kits for ZCCHC14 (MyBiosource, San Diego, CA, USA), TNF-α (Elabscience, Wuhan, Hubei, China), IL-6 (Elabscience, Wuhan, Hubei, China), and IL-10 (Beyotime, Shanghai, China) were commercially obtained. Plasma ZCCHC14, TNF-α, IL-6, and IL-10 levels were dynamically detected at different time points (days 1, 3, 7, 14, and 30 after admission) by ELISA kits according to the manufacturer's protocols and based on the quantitative sandwich enzyme immunoassay technique.

Statistical Analysis

SPSS software (Version 19.0, SPSS Inc, Chicago, IL, USA) was applied for statistical analysis. All enumeration data were provided as the mean ± standard deviation (mean ± SD) and analysed with the Kruskal-Wallis test. Binary data were analysed with the Chi-squared test. Survival differences between groups were analysed with the Gehan-Breslow-Wilcoxon test. Correlation efficiency was calculated. The p-values reported in the study were based on a two-sided probability test with a significance level of $p < 0.05$.

Results

Clinical Baseline Comparison Between ICH Patients and Healthy Controls

Baseline demographic information and clinical features are summarized in Table I. Among all patients, twenty-nine patients died within 30 days of disease onset. The remaining 82 survivors had different neurological impairments. Among these, 19 patients had severe disability, while 63 patients had mild-to-moderate disability. Furthermore, some differences in clinical characteristics were found between patients and healthy controls: hypertension (p = 0.005), angiotensin receptor blocker (ARB) application (p = 0.014), ZCCHC14 level (p < 0.001), TNF-α (p < 0.001), IL-6 (p < 0.001), and IL-10 (p < 0.001). In the multiple logistic regression analysis (Table II), the ZCCHC14 level on day 1 was significantly different between the two groups (p = 0.002, OR = 0.440, 95%CI 0.290-0.718).

Plasma ZCCHC14, TNF-α, IL-6, and IL-10 Levels Dynamically Changed Post-ICH

Plasma ZCCHC14, TNF-α, IL-6, and IL-10 concentrations on days 1, 3, 7, 14, and 30 were dynamically detected. The levels of these cytokines were elevated on day 1. The plasma levels of ZCCHC14 and IL-10 peaked on day 7, and then decreased to relatively higher levels in the patients than in the healthy controls, while the plasma levels of TNF-α and IL-6 peaked on day 3 (Figure 1).

Diagnostic Value of ZCCHC14 in ICH

We then analysed the diagnostic efficacy of plasma ZCCHC14 in ICH patients. We compared ZCCHC14 concentrations between ICH patients and healthy controls. ZCCHC14 played an excellent diagnostic role in ICH (AUC = 0.953, p < 0.0001, 95%CI: 0.901-1.004), and the cut-off concentration of ZCCHC14 was determined to be 74.50 pg/ml. The specificity and sensitivity found using ZCCHC14 in the diagnosis of ICH were 84.6% and 95.5%, respectively (Figure 2A).

Correlation Between ZCCHC14 and the Clinical Characteristics

We analysed the correlation between ZCCHC14 and the clinical characterizations of ICH patients. We found that plasma ZCCHC14 concentration was correlated with hypertension (p

Table I. Clinical epidemiological parameters for both patient group and control group.

	Patient group (n = 130) N (%)	Control group (n = 60)	*p*-value
Age (mean ± SD)	73.3 ± 6.5	72.2 ± 6.5	0.140
Gender (male)	94 (72.31)	48 (80.00)	0.257
Hypertension	72 (55.38)	46 (76.67)	**0.005**
Diabetes mellitus	21 (16.15)	17 (28.33)	0.051
Dyslipidemia	25 (19.23)	18 (30.00)	0.099
Antiplatelet use	18 (13.85)	14 (23.33)	0.104
VKA use	6 (4.62)	0 (0.00)	ND
CCB	66 (50.77)	30 (50.00)	0.921
ACEI	60 (46.15)	31 (51.67)	0.480
ARB	10 (7.69)	12 (20.00)	**0.014**
Sulfonylureas	14 (10.77)	6 (10.00)	0.872
SBP (mmHg) (IQR)	151 (132-176)	129 (113-151)	**< 0.001**
DBP (mmHg) (IQR)	77 (69-98)	67 (58-92)	0.075
ZCCHC14 (at Day 1)	91.7 ± 21.5	70.7 ± 10.8	**< 0.00**1
TNF-α (at Day 1)	21.2 ± 6.5	12.2 ± 3.9	**< 0.001**
IL-6 (at Day 1)	125.8 ± 21.9	42.3 ± 8.9	**< 0.001**
IL-10 (at Day 1)	65.2 ± 19.3	23.6 ± 5.7	**< 0.001**
GCS (IQR)	12 (7-14)	–	–
GOS (IQR)	3 (1-4)	–	–
ICH volume, cm^3 (IQR)	17.7 (11.8-79.0)	–	–
Midline shift, > 10 mm, n (%)	46 (35.38)	–	–
IVH, n (%)	57 (43.85)	–	–
Severe disability to mortality, n (%)	48 (36.92)	–	–

VKA: vitamin K antagonist; CCB: calcium channel blockers; ACEI: angiotensin-converting enzyme inhibitors; ARB: angiotensin receptor blockers; SBP: systolic blood pressure; DBP: Diastolic blood pressure; IQR: interquartile range; TNF: tumor necrosis factor; IL: interleukin; GCS: Glasgow coma scale; GOS: Glasgow outcome scale; ICH: intracerebral hemorrhage; IVH: intraventricular hemorrhage.

Table II. Logistic regression model for hypertension.

	Odd Ratio	95% Confidence Interval	*p*-value
ARB	1.112	0.733-1.852	0.219
SBP	1.065	0.309-2.571	0.190
ZCCHC14	0.440	0.290-0.718	0.002
TNF-α	1.012	0.891-5.522	0.527
IL-6	1.666	0.901-2.353	0.093
IL-10	0.655	0.212-1.400	0.106

ARB: angiotensin receptor blockers; SBP: systolic blood pressure; TNF: tumor necrosis factor; IL: interleukin.

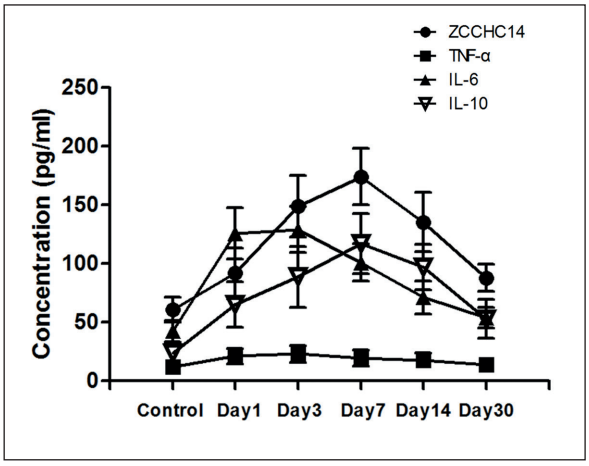

Figure 1. Dynamic screening for ZCCCH14 and inflammatory cytokines in the sICH patients.

= 0.005), GOS (p = 0.025), bleeding volume (p < 0.001), midline shift (p = 0.003), and 30-day mortality (p = 0.006) (Table III).

Prognostic Efficacy of ZCCHC14 in ICH

The patients were divided into two groups according to the plasma ZCCHC14 level on day 1 after admission. We compared the survival rates between the two groups, and the result indicated that the patients with low ZCCHC14 expression had poorer outcomes (death or severe disability) than those with high ZCCHC14 expression (Breslow p < 0.001) (Figure 2B).

Correlation Analysis Between Plasma ZCCHC14 and Concentrations of TNF-α, IL-6, and IL-10

As plasma ZCCHC14 reached its peak on day 7 after admission, we analysed the correlation between plasma ZCCHC14 and concentrations of TNF-α, IL-6, and IL-10 at that time point. The results indicated that plasma ZCCHC14 was negatively correlated with IL-6 (p = 0.024, correlation coefficient r = -0.371) and positively correlated with IL-10 (p = 0.041, correlation coefficient r = 0.298) (Table IV).

Discussion

ICH is a series of devastating cerebrovascular events with extremely high mortality. Although the diagnostic and therapeutic technologies have

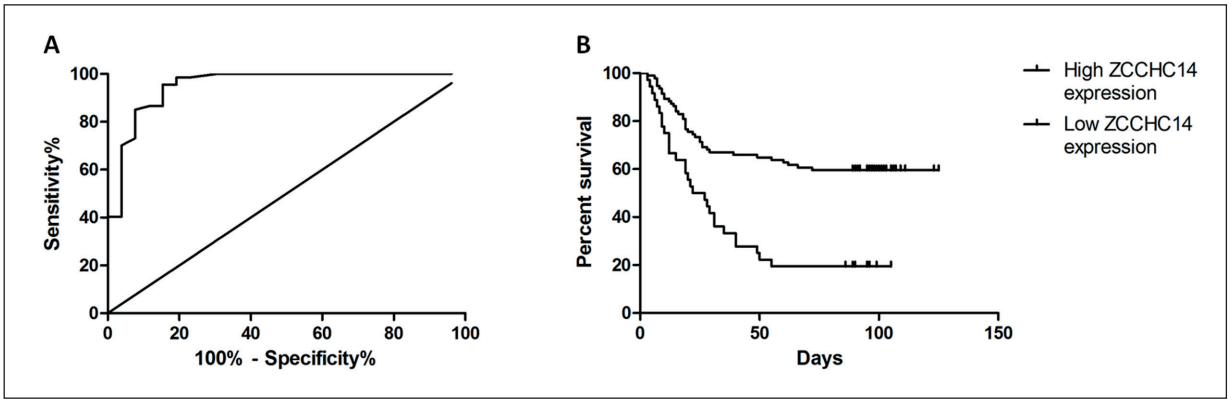

Figure 2. A, ROC curve for diagnosis of sICH from healthy control. **B,** Survival curve between low and high expression groups of ZCCHC14.

Table III. Correlation analysis of clinical epidemiological parameters between ZCCHC14 positive group and ZCCHC14 negative group.

	< 74.50 pg/ml, n = 36	≥ 74.50 pg/ml, n = 94	*p*-value
Age (mean ± SD)	72.8 ± 7.3	73.7 ± 6.2	0.257
Gender (male)	22 (61.11)	72 (76.60)	0.077
Hypertension	27 (75.00)	45 (47.87)	**0.005**
Diabetes mellitus	8 (22.22)	15 (15.96)	0.402
Dyslipidemia	8 (22.22)	19 (20.21)	0.800
Antiplatelet use	4 (11.11)	14 (14.89)	0.576
VKA use	6 (16.67)	0 (0)	ND
CCB	20 (55.56)	46 (48.94)	0.499
ACEI	14 (38.89)	46 (48.94)	0.304
ARB	0 (0)	10 (10.64)	ND
Sulfonylureas	2 (5.56)	12 (12.77)	0.235
SBP (mmHg) (IQR)	156 (135-171)	144 (126-166)	0.125
DBP (mmHg) (IQR)	75 (62-95)	79 (65-88)	0.792
GCS (IQR)	10 (7-14)	13 (11-14)	0.051
GOS (IQR)	2 (2-4)	3 (1-5)	**0.025**
sICH volume, cm^3 (IQR)	27.7 (13.0-88.5)	14.2 (10.9-43.2)	< **0.001**
Midline shift, > 10mm, n (%)	20 (55.56)	26 (27.66)	**0.003**
IVH, n (%)	30 (39.91)	16 (44.44)	0.181
Severe disability to mortality, n (%)	21 (58.33)	27 (28.72)	**0.006**

VKA: vitamin K antagonist; CCB: calcium channel blockers; ACEI: angiotensin-converting enzyme inhibitors; ARB: angiotensin receptor blockers; SBP: systolic blood pressure; DBP: Diastolic blood pressure; IQR: interquartile range; TNF: tumor necrosis factor; IL: interleukin; GCS: Glasgow coma scale; GOS: Glasgow outcome scale; ICH: intracerebral hemorrhage; IVH: intraventricular hemorrhage.

been improved in recent decades, ICH patients still suffer severe neurological impairments. Typical signs of CT and CTA, both of which are widely applied clinically, help in the rapid diagnosis of ICH. However, only 50% of progressively bleeding patients are positively detected by imageological findings[14,15]. In another aspect, molecule-based studies have not only provided important clinical clues for the precise diagnosis and outcome prediction of the disease but also investigated the pathogenesis of ICH.

ICH patients usually experience two pathologic processes: primary and secondary brain injuries[16]. Primary brain injury is caused by the mechanical damage induced by the hematoma. However, it is disappointing that effective therapeutic options for primary injury are still lacking. A surgical trial of intracerebral hemorrhage (STICH) regrettably suggested that there was no convincing evidence to support the efficacy of early surgical removal of hematomas[17]. On the other hand, in terms of the status of secondary brain injury, pathologic alterations, such as the activation of cytotoxic, excitotoxic, oxidative, and inflammatory pathways, are triggered by hematomas and result in neurologic deterioration[4,18]. Some studies have found that regulating molecules, whose levels might be altered in secondary brain injury, might promote outcomes both in clinical and animal studies. The strategies for this approach include the counterbalance of anti-inflammatory cytokines with pro-inflammatory cytokines, reduction of oxidative production, and neutralization of cytotoxic production[19,20].

Some omics-based ICH studies using high-throughput techniques have been conducted. Numerous molecules, which were considered diagnostic or prognostic markers, were validated to be dynamically changed during recovery processes. However, controversies remain regarding the clinical value of these biomarkers. A multi-centre investigation selected 21 biomark-

Table IV. Correlation of plasma ZCCHC14 level with concentrations of TNF-α, IL-6 and IL-10.

	ZCCHC14	
	r	*p*-value
TNF-α	0.133	0.432
IL-6	-0.371	**0.024**
IL-10	0.298	**0.041**

ARB: angiotensin receptor blockers; SBP: systolic blood pressure; TNF: tumor necrosis factor; IL: interleukin.

ers, which had been reported to be potentially correlated with stroke in the previous literature, and compared these biomarkers between real strokes and stroke mimics, as well as between ischaemic and hemorrhagic strokes. However, the results suggested that none of the established biomarkers had sufficient accuracy for the differential diagnosis of stroke in the hyperacute phase[21]. Therefore, the discovery of novel biomarkers is urgently needed to potentially help with clinical diagnosis and prognosis in ICH.

A recent genetic investigation on small vessel stroke (SVS) indicated that a variation of SNP rs12445022 upstream of the ZCCHC14 gene might decrease ZCCHC14 mRNA expression in arterial tissues and would therefore increase the risk of SVS13. Scholars[22,23] found that ZCCHC14 was correlated with major depressive disorder and nicotine dependence. In addition, regrettably we did not find any other studies of ZCCHC14 associated with neurological disorders and therefore could not explain the potential mechanisms of ZCCHC14 in the pathogenesis of ICH.

In our research, we found that the plasma ZCCHC14 level was significantly correlated with IL-6 and IL-10 with reverse correlation coefficients. In the pathologic processes of secondary brain injury, the comprehensive effects of both anti- and pro-inflammatory cytokines function independently and interact mutually to influence outcomes in ICH patients. In comparison with pro-inflammatory cytokines, such as IL-6 and TNF-α, IL-10 plays anti-inflammatory and neuro-protective roles in ICH by suppressing the production and activation of pro-inflammatory cytokines, as well as regulating the sensitivity of neurons towards excitatory amino acid (EAA)-induced neuro-toxicity[24]. The results indicate that ZCCHC14 potentially functions as an anti-inflammatory molecule or plays a neutralizing effect towards pro-inflammation in secondary brain injury.

There were some limitations in our study. First, the patients recruited represented a relatively small cohort and single ethnic group. Therefore, the results should be further validated in a larger cohort and in other ethnicities. Second, due to the lack of established relative studies for ZCCHC14, we did not conduct a mechanism study for the ZCCHC14 protein. This examination should be carried out in our next study to further investigate whether ZCCHC14 might exhibit neuro-protective effects by mediating anti-inflammatory effects.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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