

# Plasma SIRT3 as a Biomarker of Severity and Prognosis After Acute Intracerebral Hemorrhage: A Prospective Cohort Study

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**Objective:** SIRT3 may act as a brain-protective factor. We measured the plasma SIRT3 levels of patients with intracerebral hemorrhage (ICH) and further determined the relationship between plasma SIRT3 and clinical outcome plus severity of ICH.

**Methods:** In this prospective cohort study, we quantified plasma SIRT3 levels in 105 ICH patients and 72 healthy controls. Glasgow Coma Scale (GCS) score and hematoma volume were used to assess severity. Poor prognosis was defined as a Glasgow Outcome Scale (GOS) score of 1–3 at 90 days after ICH.

**Results:** Plasma SIRT3 levels were markedly lower in patients than in controls (median, 10.19 versus 13.17 ng/mL;  $P < 0.001$ ). Among all patients, plasma SIRT3 levels were independently correlated with hematoma volume (beta,  $-0.098$ ; 95% confidence interval,  $-0.158$ – $0.039$ ;  $t$ ,  $-3.282$ ;  $P = 0.001$ ) and GCS score (beta,  $0.465$ ; 95% confidence interval,  $0.107$ – $0.823$ ;  $t$ ,  $2.576$ ;  $P = 0.011$ ). A total of 46 cases had a poor prognosis at post-stroke 90 days. The plasma levels of SIRT3 significantly decreased in patients with a poor prognosis, compared with those with a good prognosis (median, 6.1 versus 11.2 ng/mL;  $P < 0.001$ ). Plasma SIRT3 was an independent predictor for 90-day poor prognosis of patients (odds ratio,  $0.837$ ; 95% confidence interval,  $0.708$ – $0.990$ ;  $P = 0.038$ ). Plasma SIRT3 levels distinguished the development of poor prognosis with area under receiver operating characteristic curve at  $0.801$  (95% confidence interval,  $0.711$ – $0.872$ ) and plasma SIRT3 levels  $\leq 7.38$  ng/mL predicted poor prognosis with 63.04% sensitivity and 93.22% specificity.

**Conclusion:** Declined plasma SIRT3 levels are highly associated with hemorrhagic severity and poor 90-day outcome, thus suggesting that plasma SIRT3 may serve as a potential prognostic biomarker for ICH.

**Keywords:** intracerebral hemorrhage, prognosis, severity, SIRT3, outcome

## Introduction

Intracerebral hemorrhage (ICH) is a serious public health problem with high rates of death and disability, and it accounts for 10–20% of stroke worldwide.<sup>1,2</sup> Secondary brain injury induces perihematomal edema and therefore increases intracranial pressure, finally leading to a poor prognosis after ICH.<sup>3,4</sup> The initial hematoma induces glutamate release and then leads to oxidative stress and mitochondrial dysfunction. Mitochondrial dysfunction may contribute to the deficiency of adenosine triphosphate generation and then result in the failure of cellular pumps, which causes cytotoxic edema and neuronal apoptosis.<sup>5–8</sup> Thus, inhibiting oxidative stress may be an effective method in decreasing ICH injury. In addition, some biomarkers, which are related to inflammation, oxidative stress, neuronal apoptosis and brain edema, have been extensively studied with respect to outcome prediction and severity assessment.<sup>9,10</sup> Recently, several systematic reviews and clinical studies of multiple biomarkers have shown that those biochemical markers, eg,

E-selectin, P-selectin, matrix metalloprotein-9, homocysteine and C-reactive protein, are strongly associated with clinical outcomes of ICH, but they have not been used in clinical work because of their low clinical predictive significance.<sup>11</sup> Thus, studies in search of new biomarkers are under way for aiding in ICH prognostic prediction.

The sirtuins, as a family of highly conservative NAD<sup>+</sup>-dependent enzymes, participate in transcriptional silencing and regulation of mitochondrial functions.<sup>12</sup> As one of the known seven members of the sirtuin family, SIRT3 is distinguished by its main localization in mitochondria.<sup>13–15</sup> Of note, SIRT3 obviously attenuated doxorubicin-induced reactive oxygen species (ROS) output in H9c2 cardiomyocytes.<sup>16</sup> Intriguingly, SIRT3 expression was significantly down-regulated with ROS generation in cortical neurons of rats with subarachnoid hemorrhage.<sup>17</sup> In addition, SIRT3 could effectively lessen oxidative stress and mitochondrial dysfunction after ICH in diabetic rats.<sup>18</sup> Thus, SIRT3 might play an important neuroprotective role in acute brain injury. However, up to now, no studies have focused on clinical significance of circulating SIRT3 in ICH patients. In the present study, we performed a prospective cohort study to investigate the prognostic role of plasma SIRT3 in ICH patients.

## Materials and Methods

### Study Design and Participants

A prospective cohort study was performed of patients with spontaneous ICH, who were admitted to the Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine (Hangzhou, China) between November 2018 and December 2020. All patients were consecutively enrolled according to the inclusion criteria: 1) first-ever stroke, 2) time from the onset of symptoms to admission of 24 h and 3) age of  $\geq 18$  years. We further excluded those patients with 1) hematoma resulting from congenital or acquired coagulation abnormalities, hemorrhagic transformation of cerebral infarction, moyamoya disease, intracranial aneurysm, arteriovenous malformation or tumors; 2) a surgical evacuation of hematoma; 3) primary intraventricular bleedings; 4) other previous neurological diseases, like ischemic or hemorrhagic stroke, intracranial tumors and severe head trauma; and 5) other specific diseases or conditions (eg, infection within recent a month, pregnancy, malignancies, uremia, liver cirrhosis, and chronic heart or lung disease). Simultaneously, a group of healthy individuals constituted controls. All procedures in this study were performed in accordance with the 1964 Helsinki declaration. This study was approved by the Institutional Review Board at the Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine (Opinion No: Medical Ethics Review No. 058–01. Written informed consent was acquired from patients' relatives and controls themselves.

### Recorded Variables

Upon arrival at the emergency center, demographics (age and gender), vascular risk factors (hypertension, diabetes mellitus and hyperlipidemia), medication history (use of statins, antiplatelet and anticoagulation drugs) and medical history were inquired. Glasgow Coma Scale (GCS) was utilized to evaluate stroke severity at admission. Each patient underwent a baseline head computerized tomography scan. ICH volumes were calculated using the formula  $0.5 \times a \times b \times c$ .<sup>19</sup> The presence of intraventricular and subarachnoid extension of hematoma were also recorded. Non-invasive blood pressure determinations were performed as well as systolic and diastolic arterial blood pressures were recorded upon arrival at emergency center. A Glasgow Outcome Scale (GOS) score of 1–3 at 90 days after stroke onset was assessed as a poor outcome.

### Immune Analysis

Peripheral blood samples of ICH patients were collected from 1.0 to 13.0 h (median, 6.0 h; 25th–75th percentiles, 4.5–8.3 h) after stroke and those of controls were acquired at enrollment. Blood glucose and leucocyte count at admission were determined using conventional methods. For measurement of plasma SIRT3 levels, blood samples were separated by centrifugation within 30 minutes and stored at  $-80^{\circ}\text{C}$  for subsequent assay. Plasma SIRT3 concentrations were gauged by enzyme-linked immunosorbent assay with commercially available kits (Shanghai Kexing Trading Co., Ltd) in accordance with the manufacturer's instructions. The measurements were completed by the same technician who was inaccessible to clinical information.

## Statistical Analysis

SPSS 25.0 and MedCalc 9.6.4.0 were used for statistical analysis. Figures were plotted using GraphPad Prism version 9.0. Using the Kolmogorov–Smirnov test or Shapiro–Wilk test, the normal distribution of quantitative data was assessed. They were reported as mean  $\pm$  standard deviation or medians with 25th–75th percentiles. Qualitative data were reported as counts (proportions). Data were compared among multiple groups using the Kruskal–Wallis *H*-test. Data comparisons between two groups were performed using the  $\chi^2$  test, Fisher's exact test, *t*-test, or Mann–Whitney *U*-test as appropriate. Bivariate correlations were analyzed using Spearman correlation coefficients. Afterwards, the multivariate linear regression model was built to ascertain independently correlated factors with plasma SIRT3 levels, and results were reported as beta and *t* values, which reflect the point estimate of the linear association. To identify predictors independently related to 90-day clinical outcomes, we constructed a binary logistic regression model, which contained the variables with  $P < 0.10$  in the univariate analysis. Age, intraventricular hemorrhage and infratentorial hemorrhage were not confirmed to be significantly associated with prognosis of ICH in this study, but those factors have been considered as the conventional prognostic determinants of ICH in a variety of clinical studies; thus, the preceding three factors were forced into the multivariate logistic regression model. Results were presented as odds ratios and 95% confidence intervals (CI). Receiver operating characteristic (ROC) curve analysis was performed to assess the prognostic predictive accuracy of the plasma SIRT3, and the area under the curve (AUC) was estimated. A value of  $P < 0.05$  was considered statistically significant.

## Results

### Participant Selection

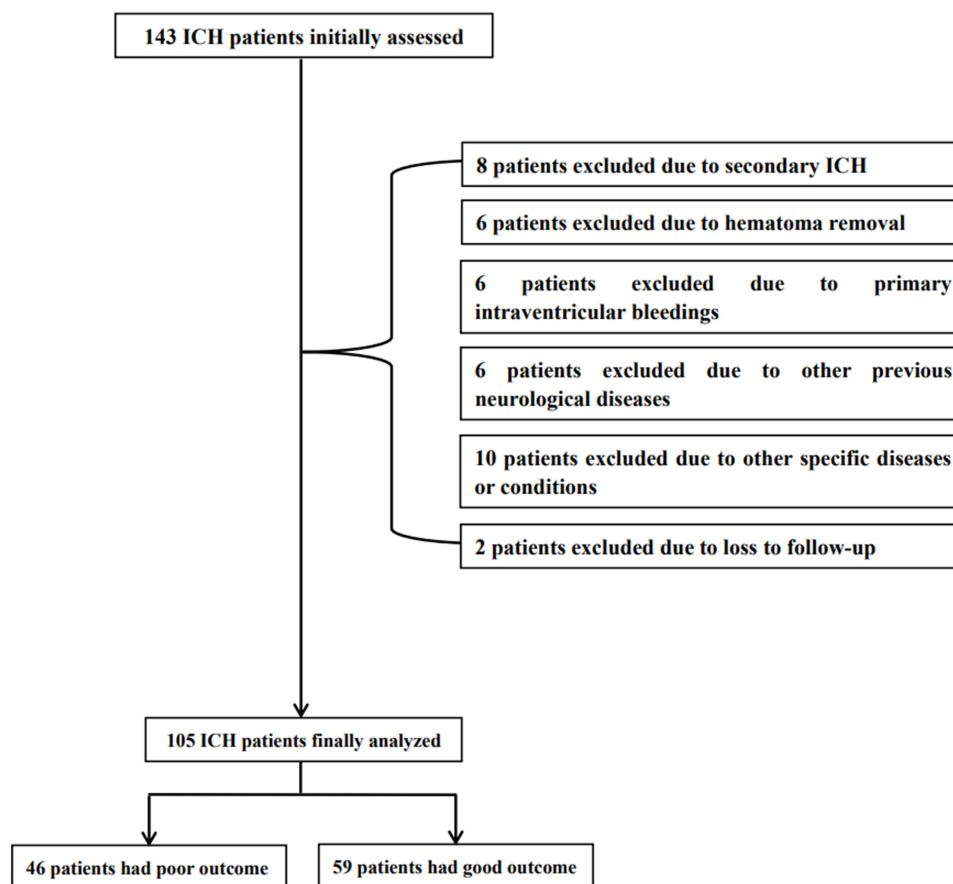
During the study, we first analyzed 143 adults with first-ever spontaneous ICH admitted within 24 hours after the onset of stroke symptoms. Then, we excluded 38 patients because of reasons outlined in [Figure 1](#). Finally, 105 ICH cases were included for further analysis. In addition, 72 healthy controls were recruited. There were no significant differences in age, sex percentage, current smoking and drinking between patients and control groups; and the proportions of hypertension, diabetes and hyperlipidemia of patients were significantly higher than those of the control group ([Table 1](#)).

### Patient Characteristics

ICH patients consisted of 69 males and 36 females, as well as their age ranged from 23 to 90 years (median, 65 years; 25th–75th percentiles, 51–76 years). In the aggregate, 27 patients smoked cigarettes and 28 patients consumed alcohol. History of past illness included hypertension (73 cases), diabetes mellitus (19 cases) and hyperlipidemia (25 cases). The patients were admitted at a median value of 5.0 h after symptom onset (range, 0.5–12.0 h; 25th–75th percentiles, 3.0–7.0 h). As regards hemorrhagic clinical severity, the median value of GCS scores was 12 (range, 4–15; 25th–75th percentiles, 10–14). With respect to some radiological parameters, hematomas of 21 cases were located at cerebral lobes; those of 19 cases were found at infratentorial cavity; intraventricular bleedings were present in 19 cases; subarachnoid hemorrhage occurred in 3 cases; and the median value of hematoma volume was 17.88 mL (25th–75th percentiles, 8.62–28.80 mL; range, 1.18–96.70 mL). Via non-invasive arterial pressure measurements, admission systolic arterial pressure and diastolic arterial pressure ranged from 91 to 234 mmHg (mean, 152.69 mmHg; standard deviation, 25.44 mmHg) and from 55 to 137 mmHg (mean, 89.08 mmHg; standard deviation, 16.49 mmHg) respectively. Laboratory tests showed that blood leucocyte count ranged from 3.0 to  $18.3 \times 10^9/l$ , with a median value of  $7.1 \times 10^9/l$  (25th–75th percentiles,  $5.6$ – $9.4 \times 10^9/l$ ), and there was a median value of 6.34 mmol/l at serum glucose levels (range, 3.92–19.10 mmol/l; 25th–75th percentiles, 5.24–8.18 mmol/l). At 90 days after stroke, GOS scores 1, 2, 3, 4 and 5 were found in 3, 14, 29, 5 and 54 patients, respectively; and GOS scores ranged from 1 to 5 (median, 5; 25th–75th percentiles, 3–5). According to the definition of poor outcome (GOS scores 1–3), a total of 46 patients suffered from a poor outcome at 90 days after stroke.

### Change of Plasma SIRT3 Levels

Among this group of ICH patients, plasma SIRT3 levels ranged from 1.02 to 17.11 ng/mL, with a median value of 10.19 ng/mL (25th–75th percentiles, 6.15–12.93 ng/mL). There was 13.17 ng/mL at the median value of plasma SIRT3 levels



**Figure 1** Flow chart for selecting eligible patients with acute spontaneous intracerebral hemorrhage. Initially, a total of 143 patients were screened, 38 were then removed, and 105 were finally included for further analysis.

**Abbreviation:** ICH, intracerebral hemorrhage.

of healthy controls (range, 4.74–20.13 ng/mL; 25th–75th percentiles, 10.38–15.93 ng/mL). Using Mann–Whitney *U*-test, it was clear that plasma SIRT3 levels were markedly lower in ICH patients than in healthy controls (Figure 2).

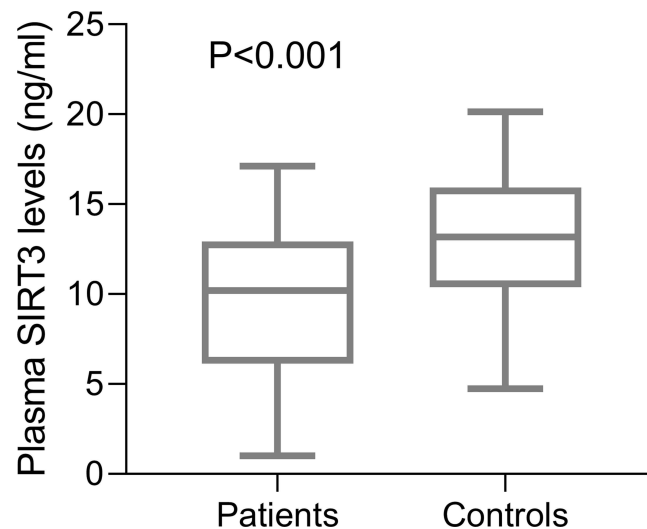
## Correlation of Plasma SIRT3 Levels with Hemorrhagic Severity

In order to verify correlations of plasma SIRT3 levels with clinical severity indicated by GCS score, GCS score was identified as a qualitative or quantitative variable. Figure 3A shows that there was a significant elevation in plasma SIRT3

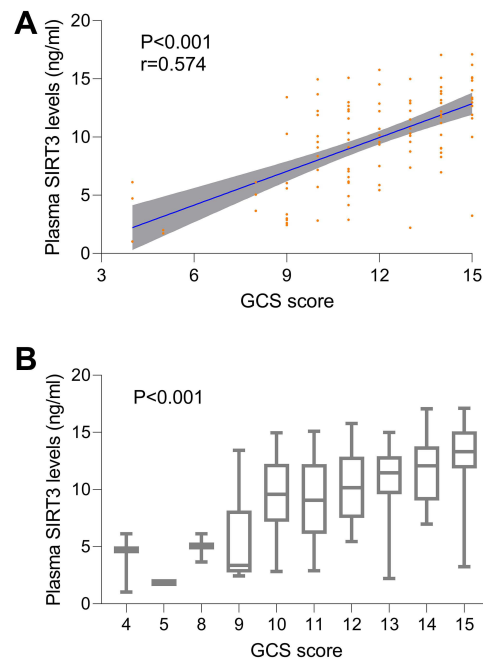
**Table 1** Comparisons of Demographic Data and Vascular Risk Factors Between Controls and Patients with Acute Intracerebral Hemorrhage

	Patients	Control	P value
Age (years)	63.6±14.7	65.8±8.6	0.379
Sex (male/female)	69/36	52/20	0.360
Hypertension	73 (69.5%)	0	<0.001
Diabetes mellitus	19 (18.1%)	0	<0.001
Hyperlipidemia	25 (23.8%)	0	<0.001
Current smoking	27 (25.7%)	14 (19.4%)	0.331
Alcohol consumption	28 (25.9%)	16 (22.2%)	0.502

**Notes:** Age was reported as the mean±standard deviation. Qualitative data were presented as counts (proportions). Intergroup comparisons of various variables were performed using the  $\chi^2$  test or Fisher's exact test for qualitative data, and Mann–Whitney *U*-test for quantitative data.



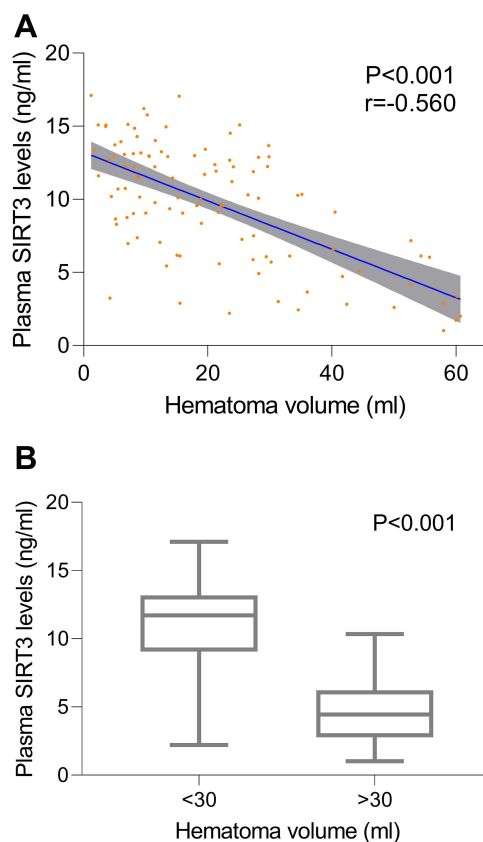
**Figure 2** Difference of plasma SIRT3 levels between healthy controls and patients with intracerebral hemorrhage. Using Mann–Whitney *U*-test, plasma SIRT3 levels were significantly lower in intracerebral hemorrhage patients than in healthy controls ( $P < 0.001$ ).



**Figure 3** Relationship between plasma SIRT3 levels and Glasgow Coma Scale score among intracerebral hemorrhage patients. **(A)** Correlative analysis of plasma SIRT3 levels with Glasgow Coma Scale score after intracerebral hemorrhage. Plasma SIRT3 levels were significantly reduced with decreased Glasgow Coma Scale score using Spearman correlation coefficients ( $P < 0.001$ ). **(B)** Differences in plasma SIRT3 levels among subgroups with different Glasgow Coma Scale score following intracerebral hemorrhage. There were statistical differences in plasma SIRT3 levels among subgroups with different Glasgow Coma Scale score, with highest levels in patients with Glasgow Coma Scale score 15 and lowest levels in those with Glasgow Coma Scale score 4 using Kruskal–Wallis *H*-test ( $P < 0.001$ ).

**Abbreviation:** GCS, Glasgow Coma Scale.

levels, with an increase in GCS score. In **Figure 3B**, there were statistical differences in plasma SIRT3 levels among subgroups with different GCS score, with highest levels in patients with GCS score 15 and lowest levels in those with GCS score 4. Alternatively, in order to demonstrate the relation of plasma SIRT3 levels to radiological severity reflected by hematoma volume, hematoma volume was considered as a quantitative variable and also was dichotomized based on the cutoff value of 30 mL. **Figure 4A** displays that the significantly negative correlations existed between plasma SIRT3



**Figure 4** Relationship between plasma SIRT3 levels and hematoma volume after intracerebral hemorrhage. **(A)** Correlative analysis of plasma SIRT3 levels with hematoma volume after intracerebral hemorrhage. Plasma SIRT3 levels were significantly, inversely correlated with hematoma volume using Spearman correlation coefficients ( $P < 0.001$ ). **(B)** Differences in plasma SIRT3 levels between two groups across hematoma volume. Patients with hematoma volume above 30 mL had substantially lower plasma SIRT3 levels than those with hematoma volume below 30 mL using Mann–Whitney *U*-test ( $P < 0.001$ ).

levels and hematoma volume, and **Figure 4B** shows that patients with hematoma volume  $\geq 30$  mL had substantially lower plasma SIRT3 levels than those with hematoma volume  $< 30$  mL.

In **Table 2**, GCS score, hematoma volume, plasma glucose levels and intraventricular hemorrhage were highly correlated with plasma SIRT3 levels. Furthermore, the preceding four significantly correlated variables were forced into the multivariate linear regression model and thereafter, it was revealed that there was an independent correlation

**Table 2** Bivariate Correlation Analysis Between Plasma SIRT3 Levels and Other Variables in 105 Intracerebral Hemorrhage Patients

Components	r	P value
Age (years)	-0.105	0.287
Sex (male/female)	0.036	0.717
Hypertension	0.077	0.432
Diabetes mellitus	-0.163	0.096
Hyperlipidemia	-0.135	0.169
Current smoking	0.066	0.505
Alcohol consumption	-0.014	0.891
Use of statins drugs	-0.013	0.895
Use of antiplatelet drugs	-0.029	0.768
Use of anticoagulation drugs	-0.077	0.433
Admission time (h)	-0.140	0.154

(Continued)

**Table 2** (Continued).

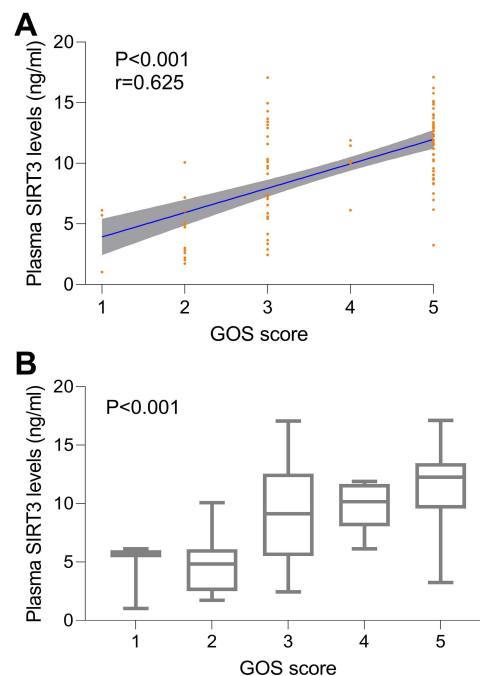
Components	r	P value
Blood-collection time (h)	-0.098	0.320
Systolic arterial pressure (mmHg)	-0.083	0.401
Diastolic arterial pressure (mmHg)	-0.057	0.563
Lobar hemorrhage	-0.094	0.339
Infratentorial hemorrhage	0.069	0.484
Intraventricular hemorrhage	-0.262	0.007
Subarachnoid hemorrhage	-0.158	0.106
Glasgow Coma Scale score	0.574	<0.001
Hematoma volume (mL)	-0.560	<0.001
Blood leucocyte count ( $\times 10^9/l$ )	-0.044	0.652
Plasma glucose levels (mmol/l)	-0.209	0.032
Plasma potassium levels (mmol/l)	-0.076	0.439
Plasma C-reactive protein levels (mg/l)	-0.113	0.249

**Note:** Correlations were done using Spearman correlation coefficient in intracerebral hemorrhage.

between plasma SIRT3 levels and GCS score (beta, 0.465; 95% CI, 0.107–0.823;  $t$ , 2.576;  $P=0.011$ ) as well as between plasma SIRT3 levels and hematoma volume (beta, -0.098; 95% CI, -0.158 to -0.039;  $t$ , -3.282;  $P=0.001$ ).

## Relationship Between Plasma SIRT3 Levels and Outcome

Just as displayed in [Figure 5A](#), plasma SIRT3 levels were remarkably increased with ascending GOS scores. And in [Figure 5B](#), substantial differences existed among subgroups with GOS scores, with highest levels in patients with GOS score 5 and lowest levels in those with GOS score 1. In [Table 3](#), plasma SIRT3 levels were substantially lower in patients with poor outcome than in those with good outcome; as compared to patients with good outcome, those presenting with



**Figure 5** Relationship between plasma SIRT3 levels and Glasgow Outcome Scale score after intracerebral hemorrhage. **(A)** Correlative analysis of plasma SIRT3 levels with Glasgow Outcome Scale score after intracerebral hemorrhage. Plasma SIRT3 levels were substantially, positively correlated with Glasgow Outcome Scale score using Spearman correlation coefficients ( $P<0.001$ ). **(B)** Differences in plasma SIRT3 levels by Glasgow Outcome Scale score after intracerebral hemorrhage. Statistical differences existed in plasma SIRT3 levels among subgroups with different Glasgow Outcome Scale score, with highest levels in patients with Glasgow Outcome Scale score 5 and lowest levels in those with Glasgow Outcome Scale score 1 using Kruskal–Wallis  $H$ -test ( $P < 0.001$ ).

**Abbreviation:** GOS, Glasgow Outcome Scale.

**Table 3** Demographic, Clinical, Radiological and Biochemical Factors for 90-Day Poor Outcome After Acute Intracerebral Hemorrhage

Components	Poor Outcome	Good Outcome	P value
Number	46 (43.8%)	59 (56.2%)	
Age (years)	65.8±15.7	61.9±13.8	0.232
Sex (male/female)	28/18	41/18	0.356
Hypertension	31 (67.3%)	42 (71.2%)	0.675
Diabetes mellitus	10 (21.7%)	9 (15.3%)	0.392
Hyperlipidemia	13 (28.3%)	12 (20.3%)	0.344
Current smoking	8 (17.4%)	19 (32.2%)	0.085
Alcohol consumption	15 (32.6%)	13 (22.0%)	0.224
Use of statins drugs	7 (15.2%)	12 (20.3%)	0.499
Use of antiplatelet drugs	5 (10.9%)	7 (11.9%)	0.874
Use of anticoagulation drugs	2 (4.3%)	1 (1.7%)	0.826
Admission time (h)	5.0 (3.0–7.3)	6.0 (3.0–7.0)	0.420
Blood-collection time (h)	5.5 (3.9–8.5)	7.0 (4.5–8.0)	0.293
Systolic arterial pressure (mmHg)	155.7±25.4	150.3±25.4	0.376
Diastolic arterial pressure (mmHg)	91.2±17.2	87.4±15.9	0.222
Lobar hemorrhage	9 (19.6%)	12 (20.3%)	0.922
Infratentorial hemorrhage	8 (17.4%)	11 (18.6%)	0.869
Intraventricular hemorrhage	10 (21.7%)	9 (15.3%)	0.392
Subarachnoid hemorrhage	2 (4.3%)	1 (1.7%)	0.826
Glasgow Coma Scale score	10 (9–11)	13 (12–14)	<0.001
Hematoma volume (mL)	29.8 (15.5–42.9)	10.5 (6.0–19.5)	<0.001
Blood leucocyte count (×10 <sup>9</sup> /l)	7.5 (5.6–10.2)	7.0 (5.5–8.9)	0.342
Plasma glucose levels (mmol/l)	7.1 (6.1–8.8)	5.7 (5.0–7.9)	0.020
Plasma potassium levels (mmol/l)	3.75±0.44	3.75±0.36	0.682
Plasma CRP levels (mg/l)	4.0 (1.6–14.9)	2.6 (2.0–5.0)	0.175
Plasma SIRT3 levels (ng/mL)	6.1 (3.6–10.2)	12.2 (9.6–13.3)	<0.001

**Notes:** Quantitative data were reported as medians with 25th–75th percentiles or the mean ± standard deviation as appropriate. Qualitative data were presented as counts (proportions). Intergroup comparisons of various variables were performed using the  $\chi^2$  test or Fisher's exact test for qualitative data, and Mann–Whitney *U*-test for quantitative data. Glasgow Outcome Scale score of 1–3 was designated as poor outcome. CRP indicates C-reactive protein.

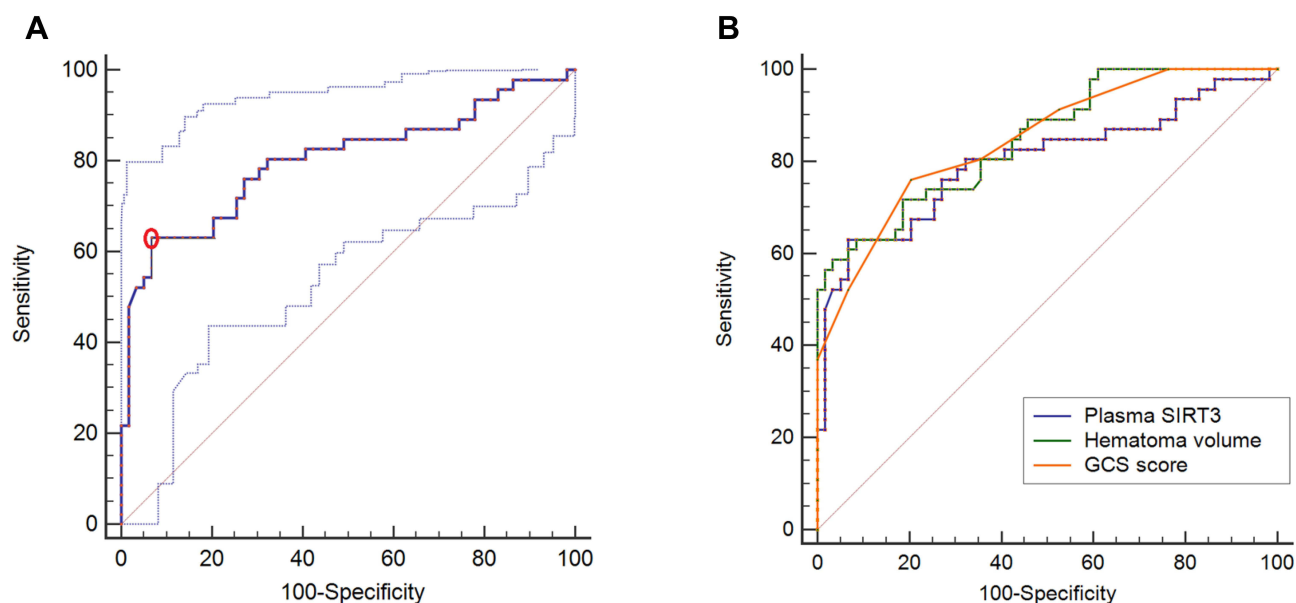
poor outcome had significantly higher hematoma volume and plasma glucose levels; and GCS score were markedly lower in patients with poor outcome than in other remainders. The variables in Table 3 with  $P < 0.10$  in univariate analysis (namely, GCS score, hematoma volume, plasma SIRT3 levels and plasma glucose levels) and other conventional prognostic determinants (namely, age, intraventricular hemorrhage and infratentorial hemorrhage) were incorporated into the binary logistic regression model, and subsequently, it was demonstrated that hematoma volume (odds ratio, 1.071; 95% CI, 1.002–1.144;  $P = 0.042$ ) and plasma SIRT3 levels (odds ratio, 0.831; 95% CI, 0.702–0.984;  $P = 0.031$ ) retained as the two independent predictors of poor outcome.

Moreover, under ROC curve, plasma SIRT3 levels remarkably predicted post-stroke 90-day poor outcome among this group of ICH patients (AUC, 0.801; 95% CI, 0.711–0.872); and plasma SIRT3 level  $\leq 7.38$  ng/mL distinguished patients with development of poor 90-day outcome with specificity and sensitivity values of 93.22% and 63.04% (Youden index *J*, 0.5626) respectively (Figure 6A). Moreover, Figure 6B shows that its discriminatory ability was equivalent to those of GCS score (AUC, 0.846; 95% CI, 0.763–0.909;  $P = 0.318$ ) and hematoma volume (AUC, 0.849; 95% CI, 0.766–0.911;  $P = 0.302$ ).

## Discussion

To the best of our knowledge, no data have been available regarding change of SIRT3 levels in the peripheral blood of patients with acute brain injury and its association with severity and prognosis. In the present study, we demonstrated that





**Figure 6** Relationship between plasma SIRT3 levels and poor outcome at 90 days after intracerebral hemorrhage. **(A)** Receiver operating characteristic curve analysis of plasma SIRT3. Plasma SIRT3 levels remarkably predicted post-stroke 90-day poor outcome (area under curve, 0.801; 95% confidence interval, 0.711–0.872). Red circle represents an optimal value of plasma SIRT3 levels, namely 7.38 pg/mL; and plasma SIRT3 levels  $\leq$  7.38 pg/mL distinguished patients with development of poor 90-day outcome with specificity and sensitivity values of 93.2% and 63.0% (maximum Youden index J, 0.5626) respectively. The two dotted lines indicates 95% confidence interval of area under curve. **(B)** Comparison of discriminatory capability with respect to plasma SIRT3 levels, Glasgow Coma Scale score and hematoma volume for 90-day poor outcome following acute intracerebral hemorrhage under receiver operating characteristic curve. Prognostic predictive ability of plasma SIRT3 level (area under curve, 0.801; 95% confidence interval, 0.711–0.872) was similar to those of Glasgow Coma Scale score (area under curve, 0.846; 95% confidence interval, 0.763–0.909;  $P=0.318$ ) and hematoma volume (area under curve, 0.849; 95% confidence interval, 0.766–0.911;  $P=0.302$ ).

**Abbreviation:** GCS, Glasgow Coma Scale.

plasma SIRT3 levels were substantially decreased in ICH patients and were highly associated with disease severity and patient prognosis. Collectively, plasma SIRT3 may be a biochemical marker of acute brain injury.

The accumulating data have shown that SIRT3 may be brain-protective via anti-oxidative activity. In an in vitro model subjected to acute glucose deprivation or acute oxygen–glucose deprivation, the overexpression of SIRT3 significantly diminished oxidative and apoptotic injury to PC12 neurons via reducing ROS and maintaining ATP production.<sup>20</sup> Also, using SIRT3 overexpressing plasmid, cortical neurons were free of excitotoxic damage induced by N-methyl-D-aspartic acid via inhibition of nicotinamide adenine dinucleotide consumption and secondary oxidative stress; nevertheless, usage of SIRT3 siRNA aggravated such damage.<sup>21</sup> Similarly, by gene knockdown or overexpression techniques, SIRT3 was proved to be protective against hypoxia or ischemia in an in vitro model of cerebral ischemia.<sup>22</sup> In line with the cytoprotective effects as a result of SIRT3 overexpression in cultured neurons, an in vivo experiment showed that SIRT3 overexpression could protect mouse cortical neurons from excitotoxicity.<sup>21</sup> Besides its anti-oxidative property, SIRT3 has shown anti-inflammatory activity in lung, liver and kidney.<sup>23–25</sup> Hence, it warrants to be studied whether SIRT3 may act as a protective factor against injured neurons via anti-inflammatory effects.

In normal cerebral cortex of rats, SIRT3 was prominently expressed in neurons.<sup>26</sup> Expression of SIRT3 protein was upregulated in in vitro-cultured neurons pretreated with N-methyl-D-aspartic acid, acute glucose deprivation or acute oxygen–glucose deprivation.<sup>20–22</sup> Maybe, the upregulated protein expression of SIRT3 in injured neurons was an endogenous feedback response for neuroprotection.<sup>20</sup> In contrast, mRNA and protein expressions of SIRT3 decreased in cortical neurons after experimental SAH.<sup>17,27</sup> Moreover, our study also found that plasma SIRT3 levels were significantly declined after ICH. It is assumed that the down-regulation of SIRT3 expression levels may be due to the depletion of neuronal function caused by toxic injury. Interestingly, the expression of brain SIRT3 was significantly decreased in diabetes ICH rats, as compared to non-diabetes ICH rats, indicating that hyperglycemia could depressed neuronal SIRT3 expression after ICH.<sup>18</sup> SIRT3 is a NAD<sup>+</sup>-dependent deacetylase.<sup>28</sup> Hyperglycemia could activate poly (ADP-ribose) polymerase, which competitively utilized the same cofactor (NAD<sup>+</sup>) with SIRT3, thereby inhibiting

expression of SIRT3.<sup>18</sup> Clearly, there is a stress-induced increase in blood glucose after acute brain injury.<sup>29</sup> Thus, it is hypothesized that the down-regulation of SIRT3 expression levels after experimental SAH and its decreased plasma levels of ICH patients in the current study may be caused by hyperglycemia after acute brain injury.

Serum SIRT3 levels were associated with 1-month mortality in patients with severe community-acquired pneumonia.<sup>30</sup> However, there is a paucity of data available regarding the relationship between circulating SIRT3 levels and illness severity in addition to clinical outcomes after acute brain injury. In this study, we found that there was an independent correlation between plasma SIRT3 levels and GCS scores, as well as between plasma SIRT3 levels and hematoma volume; in addition, plasma SIRT3 levels of ICH patients were highly correlated with GOS score. Moreover, besides hematoma volume, levels of plasma SIRT3 were independently related to the 90-day adverse prognosis (GOS score 1–3) of ICH patients. Intriguingly, plasma SIRT3 levels showed similar prognostic predictive ability, as compared to GCS scores and hematoma volume. In conclusion, plasma SIRT3 may be a promising biomarker for the prognosis of ICH.

However, there are several limitations in this study. First, this study recruited almost 100 patients and therefore there is a characteristic of small sample size. Maybe, this is a preliminary study for demonstrating the role of plasma SIRT3 in prognosis of ICH. In future, a larger cohort study is warranted to validate whether plasma SIRT3 may be a biochemical marker of acute brain injury. Second, the main goal of this study is to investigate the impact of admission plasma SIRT3 levels on ICH prognosis. Admittedly, it may be significant to observe dynamic change in plasma SIRT3 levels after stroke and further to discern the evolution of the plasma levels of SIRT3 during the follow-up period. Thus, such an investigation of circulating SIRT3 levels is warranted. Third, operation must influence postoperative levels of plasma SIRT3 levels. However, its preoperative levels are not affected by operation. Undoubtedly, operation may be related to prognosis of ICH patients. Thus, operation is a confounding factor of prognosis. In this study, we have excluded those patients undergoing a surgical evacuation of hematoma. However, it should be a good topic to determine how a surgical evacuation of hematoma may change the plasma SIRT3 levels. Fourth, accumulating evidence has shown that the role of plasma SIRT3 in acute brain injury may be protective via anti-inflammation and anti-oxidation.<sup>20–22</sup> Nevertheless, acute brain injury may be caused by a variety of underlying conditions, that reflect a wide spectrum of related pathogenetic mechanisms. Thus, further animal experiments and clinical studies are needed to discover the specific mechanisms of the protective effect of SIRT3 in acute brain injury. Fifth, clinically, many patients do not undergo a surgical operation, so direct extraction of hematoma samples is impossible. Nevertheless, plasma measurement is more convenient. However, it is undeniable that direct extraction of hematoma samples may have some clinical implications. Last, generally, GOS is frequently used to assess functional outcome of ICH, and 90-day follow-up is a conventional modality for assessing ICH prognosis.<sup>31,32</sup> Clearly, modified Rankin scale is also an alternative for function assessment of ICH.<sup>33</sup> Nevertheless, it may be of clinical value that follow-up period is extended to 6 months and even 1 year.<sup>34</sup>

## Conclusions

To the best of our knowledge, this study, for the first time, demonstrated that there is a significantly lower plasma SIRT3 levels in ICH patients than in healthy controls; and plasma SIRT3 levels had close correlations with disease severity reflected by GCS scores and hematoma volume, and independently predicted poor outcome of ICH patients. Thus, it is hypothesized that SIRT3 may be implicated in secondary brain injury after ICH and circulating SIRT3 may have the potential to represent a prognostic biochemical marker of ICH.

## Abbreviations

GCS, Glasgow Coma Scale; ICH, intracerebral hemorrhage; CT, computerized tomography; ROC, receiver operating characteristic; AUC, area under curve; 95% CI, 95% confidence interval; ROS, reactive oxygen species.

## Data Sharing Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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## Disclosure

The authors have no conflicts of interest in this work.

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