

# Utility of Serum Growth Arrest-Specific Protein 6 as a Biomarker of Severity and Prognosis After Severe Traumatic Brain Injury: A Prospective Observational Study

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**Objective:** Growth arrest-specific protein 6 (Gas6) may harbor protective effects in acute brain injury. This study was designed to determine the relation of serum Gas6 levels to severity and prognosis after traumatic brain injury (TBI).

**Methods:** In this prospective cohort study of 114 controls and 114 patients with severe TBI, multivariate analysis was used to assess relationships between serum Gas6 levels, Glasgow coma scale (GCS) score, Rotterdam computed tomography (CT) score, postinjury 180-day mortality, overall survival and poor prognosis (Extended Glasgow outcome scale score 1–4).

**Results:** Significantly increased serum Gas6 levels of patients (median, 10.3 ng/mL versus 32.5 ng/mL;  $P < 0.001$ ), as compared with controls, were independently correlated with Rotterdam CT score ( $t = 3.629$ ,  $P < 0.001$ ) and GCS score ( $t = -3.393$ ,  $P = 0.001$ ), and independently predicted 180-day mortality (odds ratio, 1.078; 95% confidence interval (CI), 1.007–1.154), overall survival (hazard ratio, 1.074; 95% CI, 1.012–1.139) and poor prognosis (odds ratio, 1.129; 95% CI, 1.059–1.205). Areas under receiver operating characteristic curve (AUCs) of serum Gas6 levels for discriminating risks of 180-day mortality and poor prognosis were 0.785 (95% CI, 0.699–0.857) and 0.793 (95% CI, 0.707–0.863), respectively; and serum Gas6 levels above 30.9 ng/mL and 28.3 ng/mL predicted 180-day mortality and poor prognosis with maximum Youden indices of 0.451 and 0.468, respectively. The predictive ability of serum Gas6 levels for mortality was similar to those of GCS score (AUC, 0.833; 95% CI, 0.751–0.896;  $P = 0.286$ ) and Rotterdam CT score (AUC, 0.823; 95% CI, 0.740–0.888;  $P = 0.432$ ). The discriminatory capability of serum Gas6 levels for the risk of poor prognosis was in the range of GCS score (AUC, 0.846; 95% CI, 0.766–0.906;  $P = 0.178$ ) and Rotterdam CT score (AUC, 0.831; 95% CI, 0.750–0.895;  $P = 0.368$ ).

**Conclusion:** Serum Gas6 may appear as a promising biochemical parameter for aiding in the assessment of trauma severity and prediction of prognosis among patients with severe TBI.

**Keywords:** traumatic brain injury, biomarkers, growth arrest-specific protein 6, severity, prognosis

## Introduction

Severe traumatic brain injury (sTBI) is one of the most devastating types of trauma associated with high morbidity and mortality rates.<sup>1,2</sup> The Glasgow coma scale (GCS) is a clinically valuable prognostic factor of TBI.<sup>3–5</sup> Secondary brain injury after TBI involves inflammation, oxidative stress, cellular death, excitatory amino acids toxicity and so on.<sup>6–8</sup> In recent decades, some relevant biochemical markers, such as S100B, glial fibrillary acidic protein, ubiquitin carboxyl-terminal hydrolase L1, myelin basic protein and neuron-specific enolase, have obtained interests of neurological researchers for helping clinical prognostic prediction and severity assessment of TBI.<sup>9–11</sup> However, circulating levels of those biomarkers have not been conventionally determined for clinical service. Hence, clinical investigation of potential biomarkers is still under way for severity and prognosis analysis of TBI.

Vitamin K-dependent proteins (VKDPs) are a group of proteins, which need vitamin K to conduct carboxylation.<sup>12</sup> Tyro3, Axl and MerTK are collectively termed as TAMs, which are tyrosine kinase cell surface transmembrane receptors

and are capable of sensing extracellular ligands, phosphorylating and activating diverse downstream signaling pathways.<sup>13</sup> Growth arrest-specific protein 6 (Gas6), which is identified as a member of the VKDP family, can bind the TAM receptors.<sup>14</sup> Gas6/TAM signaling may play an important role in apoptotic cell clearance, immune homeostasis and resolution of immune responses.<sup>15,16</sup> Gas6 can be extensively expressed in neurons, glial cells and endothelial cells of the central nervous system.<sup>17,18</sup> Compelling experimental data have shown that Gas6 facilitated the clearance of apoptotic bodies,<sup>19</sup> and inhibited neuroinflammation after acute brain injury,<sup>20,21</sup> indicating its brain-protective effects. Interestingly, elevated Gas6 levels in cerebrospinal fluid were intimately correlated with disease duration and severity of Alzheimer's disease.<sup>22</sup> Hence, it has been hypothesized that Gas6 may be a potential biomarker for acute brain injury. In this study, we intended to explore the relationship between serum Gas6 levels and the severity plus outcome after sTBI.

## Materials and Methods

### Study Design and Participant Selection

In this prospective cohort study performed at the Wenzhou Central Hospital from January 2018 to January 2021, we initially assessed adult (age  $\geq 18$  years) patients with isolated and blunt sTBI (GCS score of 3–8), who were hospitalized within post-traumatic twelve hours. Isolated head trauma was referred to as injury severity score below 9 in non-cranial aspects. The exclusion criteria were as follows: 1) history of neurological diseases, for example, hemorrhagic or ischemic stroke, intracranial tumors and severe head trauma; 2) other specific conditions or diseases, for instance severe infections within a month, autoimmune diseases, severe liver, pregnancy, kidney or heart diseases and other known malignancies; 3) loss to follow-up, incomplete information, unavailable blood samples or refusal to participation. Simultaneously, healthy volunteers were enrolled as controls. The current study conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the Institutional Review Committee at the Wenzhou Central Hospital (No. WCH2017014). The informed consent to participate in this study was signed by the legal representatives of patients and controls themselves.

### Data Collection, Outcome Assessment and Biochemical Measurements

Some pertinent information was registered, which included age, gender, cigarette smoking, alcohol consumption, hypertension, diabetes mellitus, indicators of traumatic severity, radiological parameters, traumatic mechanisms, vital signs and clinical outcome. Alcohol abuse was defined as moderate-to-severe alcohol consumption in social history ( $>168\text{g/week}$ ).<sup>23</sup> Current cigarette smokers were those who smoked regularly at least one cigarette per day at the time of presentation.<sup>23</sup> Hypertension and diabetes mellitus were previously diagnosed. Vital signs included heart rate, respiratory rate, blood oxygen saturation and arterial blood pressure. Radiological appearances included abnormal cisterns, midline shift, epidural hematoma, subdural hematoma, subarachnoid hemorrhage, intraventricular hemorrhage, intracerebral hematoma, brain contusion and pneumocephalus. Traumatic mechanisms included automobile/motorcycle, fall/jump and others. Clinical severity was evaluated using GCS and radiological severity was assessed utilizing Rotterdam computed tomography (CT) classification. We used 8-grade Extended Glasgow Outcome Scale (GOSE) to assess clinical outcome at 180 days after head trauma. Patients with GOSE score of 1–4 was considered to have a poor outcome.<sup>24</sup>

Venous blood samples from controls and patients were obtained and centrifuged thereafter. Acquired serum was promptly preserved at  $-80^{\circ}\text{C}$  until measurements. Serum Gas6 levels were in duplicate quantified using a commercially available enzyme-linked immunosorbent assay kit based on the manufacturer's instructions (Shanghai Kexing Trading Co., Ltd., Shanghai, China). Measurements were completed by the same technician blinded to clinical information.

### Statistical Analysis

Data analyses were performed using the SPSS 20.0 statistical package (SPSS Inc., Chicago, Illinois, USA), except for the receiver operating characteristic (ROC) curve analysis, which was completed via MedCalc statistical software version 17.4 (MedCalc Software, Mariakerke, Belgium). After the normal distribution test, quantitative data were reported as means (standard deviations) if normally distributed and as medians (upper – lower quartiles) if non-normally distributed. Qualitative data were summarized as counts (percentages). Normally distributed data were compared between two groups using the independent *t*-test and non-normally distributed data were compared using the Mann–Whitney test. Intergroup

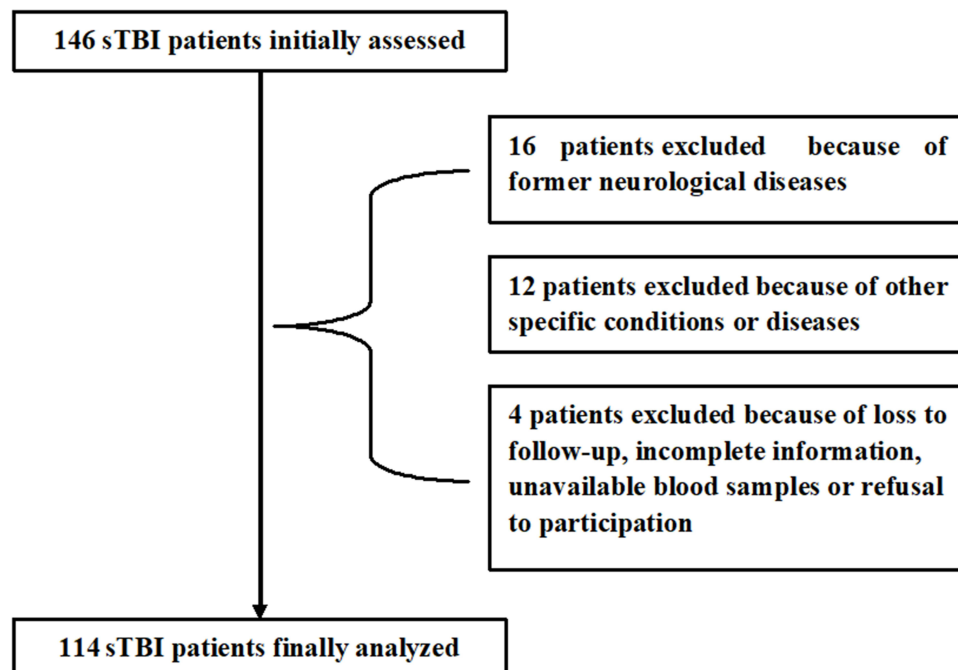
comparisons of categorical data were done using the Pearson's Chi-square test or Fisher's exact test as appropriate. The Kruskal–Wallis H-test was done to compare non-normally distributed data among multiple groups. The Log rank test was carried out to compare 180-day overall survival time between the two groups. The ROC curve was drawn to assess the predictive ability of serum Gas6 levels for mortality and poor prognosis, and the area under ROC curve (AUC) was generated to reflect the predictive efficiency. Variables, which were statistically significantly different using univariate analysis, were forced into the multivariate binary logistic regression model or multivariate Cox's proportional hazard model to determine independent predictors substantially associated with 180-day mortality, poor prognosis or overall survival. Results were reported as odds ratio (OR) or hazard ratio (HR), with the corresponding 95% confidence interval (95% CI). For all tests, the two-sided P-values less than 0.05 were considered statistically significant.

## Results

### Patient and Control Characteristics

During the study period, we firstly enrolled 146 patients with sTBI and thereafter 32 sTBI patients were excluded for the reasons outlined in Figure 1. The remaining 114 patients consisted of 68 males and 46 females, as well as were aged from 18 to 73 years (mean, 43.6 years; standard deviation, 14.0 years). The excluded 32 patients comprised 17 males and 15 females, as well as had a mean age of 45.0 years (range, 21–76 years; standard deviation, 14.3 years). There were no significant differences in terms of age and gender percentage between the finally assessed patients and the excluded patients (both  $P > 0.05$ ). Meanwhile, there were 114 healthy controls, who were aged from 18 to 77 years (mean, 44.2 years; standard deviation, 13.8 years), and included 59 males and 55 females. As compared with the eligible patients, controls had similar age and proportion of gender (both  $P > 0.05$ ).

This group of sTBI patients were analyzed for their related data. There were 68 males and 46 females, and the ratio of male to female was 1.48. They had a mean age of 43.6 years (standard deviation, 14.0 years; range, 18–73 years). There were 35 cigarette smokers, 37 alcohol drinkers, 19 hypertensive patients, 14 patients with diabetes mellitus and 22 patients with hyperlipidemia. Patients were admitted to hospital from 0.5 to 12.0 hours after head trauma (median, 4.7 hours; percentiles 25th–75th, 3.8–6.4 hours). Blood samples were obtained from 1.0 to 14.0



**Figure 1** Flowing-chart for selecting eligible patients with severe traumatic brain injury. Initially, 146 patients with severe traumatic brain injury were assessed and then 32 patients were excluded according to the exclusion criteria. Ultimately, 114 patients were analyzed. sTBI denotes severe traumatic brain injury.

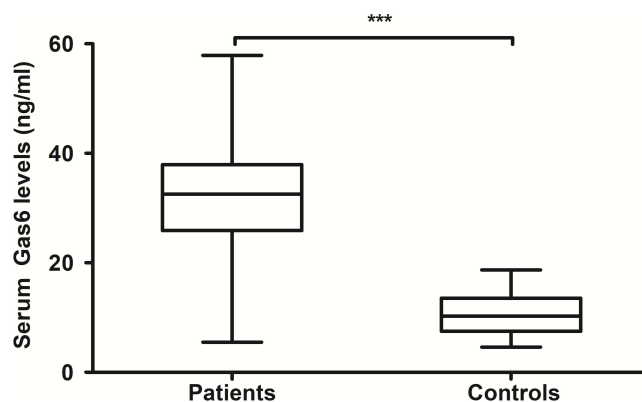
hours (median, 6.4 hours; percentiles 25th–75th, 4.6–8.0 hours) following head trauma. Traumatic causes included automobile/motorcycle (59 cases), fall/jump (44 cases) and others (11 cases). GCS score ranged from 3 to 8, with a median value of 5 and upper-lower quartiles of 4–6. GCS scores 3, 4, 5, 6, 7 and 8 were found in 14, 26, 30, 16, 13 and 15 patients, respectively. The mean systolic and diastolic arterial pressures were 124.9 mmHg (standard deviation, 28.4 mmHg; range, 74–178 mmHg) and 74.1 mmHg (standard deviation, 16.5 mmHg; range, 44–108 mmHg), respectively. Rotterdam CT score ranged from 3 to 6 (median, 4; percentiles 25th–75th, 4–5). Rotterdam CT scores 3, 4, 5 and 6 were revealed among 14, 54, 26 and 20 cases, respectively. Positive radiological appearances included abnormal cisterns (87 cases), midline shift (71 cases), epidural hematoma (61 cases), subdural hematoma (66 cases), subarachnoid hemorrhage (74 cases), intraventricular hemorrhage (12 cases), intracerebral hematoma (65 cases), brain contusion (69 cases) and pneumocephalus (43 cases). Blood glucose levels ranged from 2.8 to 19.7 mmol/l (median, 7.8 mmol/l; lower – upper quartiles, 6.1–10.4 mmol/l). Blood leucocyte count ranged from 3.4 to  $18.3 \times 10^9/l$  (median,  $8.5 \times 10^9/l$ ; lower-upper quartiles,  $6.3$ – $10.4 \times 10^9/l$ ).

## Serum Gas6 Levels and Trauma Severity

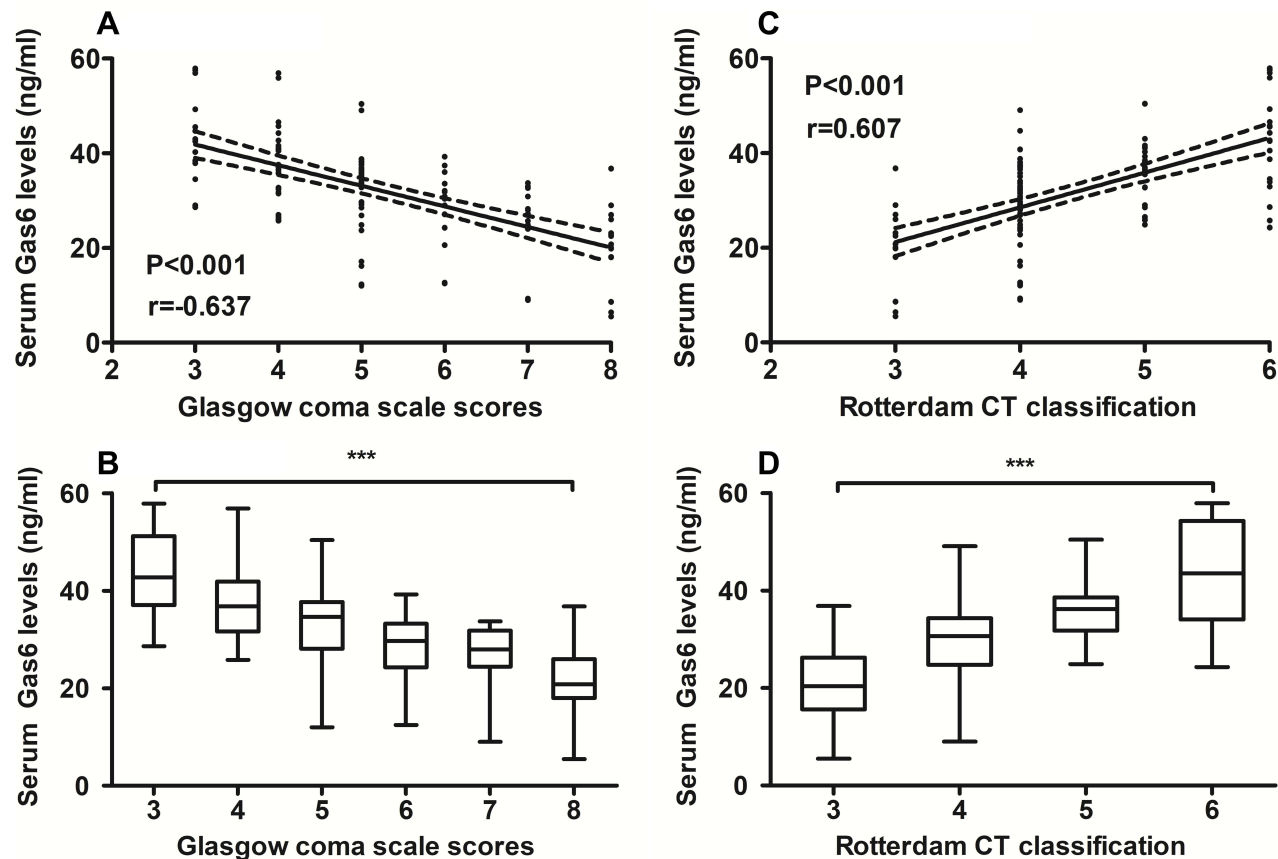
In Figure 2, serum Gas6 levels were substantially higher in sTBI patients than in healthy controls. Regarding the relationship between serum Gas6 levels and traumatic severity indicated by GCS score and Rotterdam CT score, we considered both GCS score and Rotterdam CT score as two categorical or continuous variables. Figure 3A and B show that there was a significant increase in serum Gas6 levels with a decrease in GCS score, and Figure 3C and D display that serum Gas6 levels were substantially increased with rising Rotterdam CT score. Moreover, in Table 1, serum Gas6 levels were highly correlated not only with GCS score and Rotterdam CT score but also with other variables, including abnormal cisterns, midline shift >5 mm, epidural hematoma, subdural hematoma and blood glucose levels. When the preceding significantly correlated variables were incorporated into the multivariate linear regression model, it was demonstrated that serum Gas6 levels were independently correlated with GCS score ( $t = -3.393$ ,  $P = 0.001$ ) and Rotterdam CT score ( $t = 3.629$ ,  $P < 0.001$ ).

## Serum Gas6 Levels and 180-Day Mortality

Among this cohort of sTBI patients, a total of 28 patients were deceased within 180 days following head trauma. In Figure 4A, serum Gas6 levels markedly differed between non-survivors and survivors; and moreover, serum Gas6 levels efficiently discriminated the risk of 180-day death (Figure 4B). In Table 2, the variables, which were significantly associated with 180-day death using univariate analysis, included GCS score, Rotterdam CT classification, abnormal cisterns, midline shift >5 mm, epidural hematoma, blood glucose levels and serum Gas6 levels. When the preceding significant variables were forced into the binary logistic regression model, it was confirmed that GCS score (OR, 0.502; 95% CI, 0.265–0.948), Rotterdam CT score (OR, 3.180; 95% CI, 1.360–7.437) and serum Gas6 levels (OR, 1.078; 95%



**Figure 2** Difference in terms of serum growth arrest-specific protein 6 levels between controls and patients with severe traumatic brain injury. Serum growth arrest-specific protein 6 levels were significantly higher in patients than in controls (\*\*\* $P < 0.001$ ). Gas6 indicates growth arrest-specific protein 6.



**Figure 3** Relationship between serum growth arrest-specific protein 6 levels and traumatic severity in patients with severe traumatic brain injury. **(A)** Relationship between serum growth arrest-specific protein 6 levels and Glasgow coma scale scores in patients with severe traumatic brain injury. **(B)** Comparisons of serum growth arrest-specific protein 6 levels among subgroups with different Glasgow coma scale scores in patients with severe traumatic brain injury. **(C)** Relationship between serum growth arrest-specific protein 6 levels and Rotterdam computed tomography scores in patients with severe traumatic brain injury. **(D)** Comparisons of serum growth arrest-specific protein 6 levels among subgroups with different Rotterdam computed tomography scores in patients with severe traumatic brain injury. Whether Glasgow coma scale score and Rotterdam computed tomography score were considered as a continuous or categorical variable, their close correlations still existed (\*\*\* $P < 0.001$ ).

**Abbreviations:** Gas6, growth arrest-specific protein 6; CT, computed tomography.

CI, 1.007–1.154) were independently associated with post-traumatic 180-day death. Under ROC curve, the predictive ability of serum Gas6 levels was similar to those of GCS score (AUC, 0.833; 95% CI, 0.751–0.896;  $P = 0.286$ ) and Rotterdam CT score (AUC, 0.823; 95% CI, 0.740–0.888;  $P = 0.432$ ).

### Serum Gas6 Levels and 180-Day Overall Survival

This group of sTBI patients had the mean 180-day overall survival time of 144.1 days (95% CI, 131.9–156.4 days). Based on the cutoff value of serum Gas6 levels, which was yielded using the Youden method under the ROC curve for predicting 180-day death (Figure 4B), all patients were dichotomized into two groups with serum Gas6 levels  $>$  and  $\leq 30.9$  ng/mL respectively. In Figure 4C, patients with serum Gas6 levels  $> 30.9$  ng/mL had substantially shorter 90-day overall survival time than the other remainders. Just as listed in Table 3, GCS score, Rotterdam CT classification, abnormal cisterns, midline shift  $> 5$  mm, epidural hematoma, blood glucose levels and serum Gas6 levels were substantially related to 180-day overall survival using univariate Cox's proportional hazard model. Furthermore, the afore-mentioned significant relevant variables were entered in multivariate Cox's proportional hazard model and thereafter, it was revealed that GCS score, Rotterdam CT score and serum Gas6 levels independently predicted 180-day overall survival with HR values of 0.630 (95% CI, 0.401–0.992), 2.285 (95% CI, 1.243–4.200) and 1.074 (95% CI, 1.012–1.139), respectively.

**Table 1** Bivariate Correlation Analysis of Serum Growth Arrest-Specific Protein 6 Levels After Severe Traumatic Brain Injury

	<b>r</b>	<b>P value</b>
Gender (male/female)	−0.015	0.875
Age (y)	0.075	0.425
Current cigarette smoking	0.161	0.087
Alcohol abuse	0.092	0.329
Hypertension	0.089	0.344
Diabetes mellitus	0.138	0.144
Hyperlipidemia	0.009	0.923
Admission time after trauma (h)	−0.106	0.261
Time from trauma to sampling (h)	−0.124	0.190
Traumatic causes	−0.056	0.554
Glasgow coma scale score	−0.637	<0.001
Systolic arterial pressure (mmHg)	−0.181	0.054
Diastolic arterial pressure (mmHg)	−0.090	0.344
Rotterdam CT classification	0.607	<0.001
Abnormal cisterns	0.198	0.034
Midline shift > 5 mm	0.321	0.001
Epidural hematoma	0.310	0.001
Subdural hematoma	0.199	0.034
Subarachnoid hemorrhage	0.129	0.172
Intraventricular hemorrhage	0.083	0.378
Intracerebral hematoma	0.037	0.707
Brain contusion	0.110	0.244
Pneumocephalus	0.026	0.780
Blood glucose levels (mmol/l)	0.250	0.007
Blood leucocyte count ( $\times 10^9/l$ )	0.090	0.340

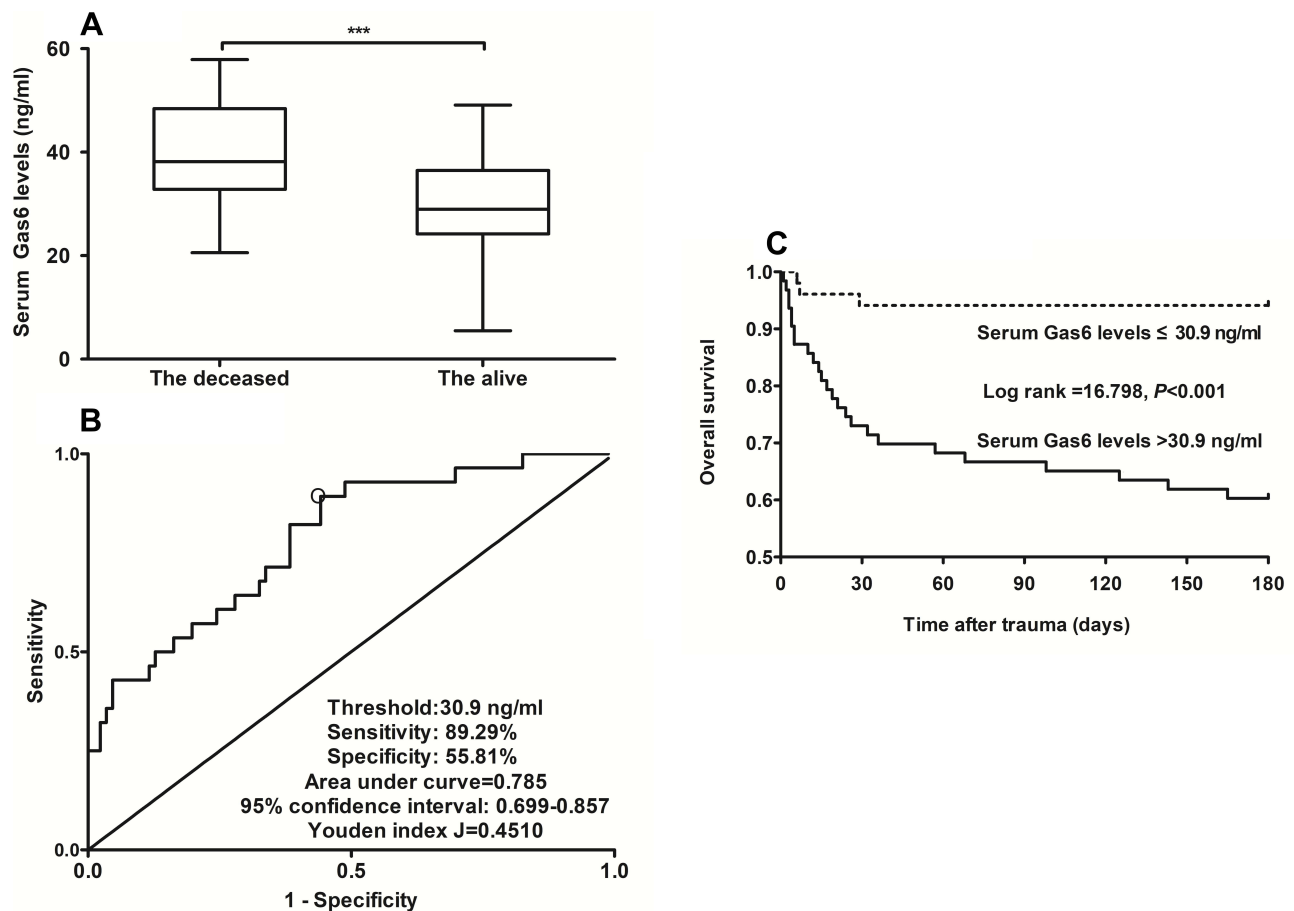
**Note:** Bivariate correlations were done using Spearman test.

**Abbreviation:** CT, computerized tomography.

## Serum Gas6 Levels and 180-Day Poor Outcome

At 180 days after head trauma, GOSE scores ranged from 1 to 8 (median, 5; percentiles 25th–75th, 2–6); and GOSE scores 1, 2, 3, 4, 5, 6, 7 and 8 were found in 28, 10, 6, 8, 24, 18, 11 and 9 patients, respectively. In total, 52 patients experienced a poor outcome (GOSE scores 1–4). [Figure 5A](#) and [B](#) display that serum Gas6 levels were tightly and inversely correlated with GOSE scores. Just as depicted in [Figure 5C](#), serum Gas6 levels were significantly higher in patients with the development of poor outcome than in those presenting with good outcome; alternatively, serum Gas6 levels substantially distinguished patients at the risk of poor outcome at post-traumatic 180 days ([Figure 5D](#)). In [Table 4](#), as compared to patients who presented with good outcome, those suffering from poor outcome tended to have significantly declined GCS score, were more likely to display substantially increased Rotterdam CT score, blood glucose levels and serum Gas6 levels, and were more prone to exhibit markedly elevated percentages of diabetes mellitus, abnormal cisterns, midline shift >5 mm, subdural hematoma, subarachnoid hemorrhage and epidural hematoma. When the above-mentioned significant variables were contained into the binary logistic regression model, it was shown that factors independently associated with 180-day poor outcome were GCS score (OR, 0.485; 95% CI, 0.267–0.880), Rotterdam CT score (OR, 6.159; 95% CI, 2.242–16.919) and serum Gas6 levels (OR, 1.129; 95% CI, 1.059–1.205). Under ROC curve, the discriminatory capability of serum Gas6 levels was in the range of GCS score (AUC, 0.846; 95% CI, 0.766–0.906;  $P = 0.178$ ) and Rotterdam CT score (AUC, 0.831; 95% CI, 0.750–0.895;  $P = 0.368$ ).





**Figure 4** Relationship between serum growth arrest-specific protein 6 levels and posttraumatic 180-day mortality in patients with severe traumatic brain injury. **(A)** Comparisons of serum growth arrest-specific protein 6 levels between the deceased and the alive. **(B)** Serum growth arrest-specific protein 6 levels with respect to predictive ability for 180-day mortality. **(C)** 180-day overall survival curve for comparing survival time and probability between subgroups with serum growth arrest-specific protein 6 levels above or below cutoff value under receiver operating characteristic curve among severe traumatic brain injury patients. Serum growth arrest-specific protein 6 levels of non-survivors were substantially higher than those of survivors (\*\*\* $P < 0.001$ ). Serum growth arrest-specific protein 6 levels efficiently discriminated the risk of 180-day mortality ( $P < 0.001$ ). Overall survival time was significantly shorter in patients with serum growth arrest-specific protein 6 levels  $> 30.9$  ng/mL than the other remainders ( $P < 0.001$ ). Gas6 indicates growth arrest-specific protein 6.

## Discussion

To the best of our knowledge, this is the first series for measuring circulating Gas6 levels in humans after acute brain injury and further determining the relation of serum Gas6 levels to trauma severity and clinical outcome after sTBI. The main findings are that (1) there was a significant elevation in serum Gas6 levels of patients after sTBI; (2) serum Gas6 levels were independently correlated with GCS score and Rotterdam CT score; (3) serum Gas6 levels were independently associated with 180-day death, overall survival and poor outcome; and (4) serum Gas6 levels possessed similar AUCs, as compared to GCS score and Rotterdam CT score. Presumably, serum Gas6 levels should be intimately correlated with trauma severity and be highly associated with clinical outcome after sTBI.

Gas6 is a multifunctional ligand of TAM receptors Tyro3, Axl and Mertk, with the highest affinity to Axl.<sup>14</sup> TAM receptors are extensively expressed in the nervous system.<sup>25</sup> And Gas6 is abundantly secreted by neurons and endothelial cells.<sup>17,18</sup> In vitro experiment showed that the administration of recombinant Gas6 significantly alleviated hippocampal neuronal cell death caused by serum starvation.<sup>19</sup> Similarly, in mice with subarachnoid hemorrhage, administration of recombinant Gas6 markedly decreased expressions of interleukin-1 $\beta$  and interleukin-18, lessened brain edema, reduced neuronal degeneration and diminished neurological deficits.<sup>21</sup> Alternatively,

**Table 2** Factors Associated with 180-Day Mortality After Severe Traumatic Brain Injury

	Non-Survivors	Survivors	P value
Gender (male/female)	17/11	51/35	0.895
Age (y)	45.5±15.5	43.0±13.6	0.403
Current cigarette smoking	11 (39.3%)	24 (27.9%)	0.257
Alcohol abuse	11 (39.3%)	26 (30.2%)	0.374
Hypertension	7 (25.0%)	12 (14.0%)	0.241
Diabetes mellitus	6 (21.4%)	8 (9.3%)	0.104
Hyperlipidemia	6 (21.4%)	16 (18.6%)	0.742
Admission time after trauma (h)	4.5 (3.0–5.8)	4.8 (4.0–6.4)	0.193
Time from trauma to sampling (h)	5.4 (3.9–7.6)	6.5 (5.0–8.3)	0.168
Traumatic causes			0.368
Automobile/motorcycle	16	43	
Fall/jump	8	36	
Others	4	7	
Glasgow coma scale score	4 (3–5)	6 (5–7)	<0.001
Systolic arterial pressure (mmHg)	118.8±26.1	126.8±29.1	0.195
Diastolic arterial pressure (mmHg)	72.8±15.9	74.4±16.7	0.662
Rotterdam CT classification	6 (5–6)	4 (4–5)	<0.001
Abnormal cisterns	26 (92.9%)	61 (70.9%)	0.018
Midline shift > 5 mm	23 (82.1%)	48 (55.8%)	0.013
Epidural hematoma	20 (71.4%)	41 (47.7%)	0.029
Subdural hematoma	20 (71.4%)	46 (53.5%)	0.095
Subarachnoid hemorrhage	21 (75.0%)	53 (61.6%)	0.198
Intraventricular hemorrhage	5 (17.9%)	7 (8.1%)	0.164
Intracerebral hematoma	19 (67.9%)	46 (53.5%)	0.182
Brain contusion	18 (64.3%)	51 (59.3%)	0.639
Pneumocephalus	12 (42.9%)	31 (36.1%)	0.518
Blood glucose levels (mmol/l)	9.1 (7.5–11.3)	7.4 (5.7–9.9)	0.006
Blood leucocyte count ( $\times 10^9/l$ )	8.8 (6.5–11.2)	7.7 (6.0–9.8)	0.314
Serum Gas6 levels (ng/mL)	38.2 (32.9–47.5)	29.0 (24.3–36.4)	<0.001

**Notes:** Quantitative data were presented as mean  $\pm$  standard deviation if normally distributed and as median (25th–75th percentiles) if non-normally distributed. Qualitative data were reported as counts (percentages). Intergroup comparisons of data were done using the t-test, Mann–Whitney U-test, Pearson chi-square test or Fisher's exact test as appropriate.

**Abbreviations:** CT, computerized tomography; Gas6, growth arrest-specific protein 6.

expressions of Gas6 were greatly up-regulated in mouse brain tissues after intracerebral hemorrhage, as well as administration of recombinant Gas6 significantly diminished brain edema and improved neurobehavioral performances.<sup>20</sup> Also, in animal studies, administration of recombinant Gas6 significantly reduced brain infarction, diminished neurological deficits, attenuated blood–brain barrier disruption and depressed hemorrhagic transformation after intravenous thrombolysis.<sup>26</sup> Overall, rapidly growing evidence has been supportive of the notion that Gas6 may act as a brain-protective protein.

Circulating Gas6 levels have been found to be significantly increased in some critically ill patients with COVID-19,<sup>27</sup> sepsis<sup>28</sup> or acute myocardial infarction.<sup>29</sup> However, the current study has excluded such critically ill patients from our cohort. Gas6 can be produced greatly from injured brain tissues.<sup>20</sup> Thus, it is hypothesized that Gas6 may be released into peripheral blood via disrupted blood–brain barrier after head trauma. So, blood Gas6 may be at least partly derived from injured brain tissues. On the other hand, the release of Gas6 from traumatized brain tissues may be a compensatory increase in order to exert a brain-protective effect.



**Table 3** Factors Associated with 180-Day Overall Survival After Severe Traumatic Brain Injury

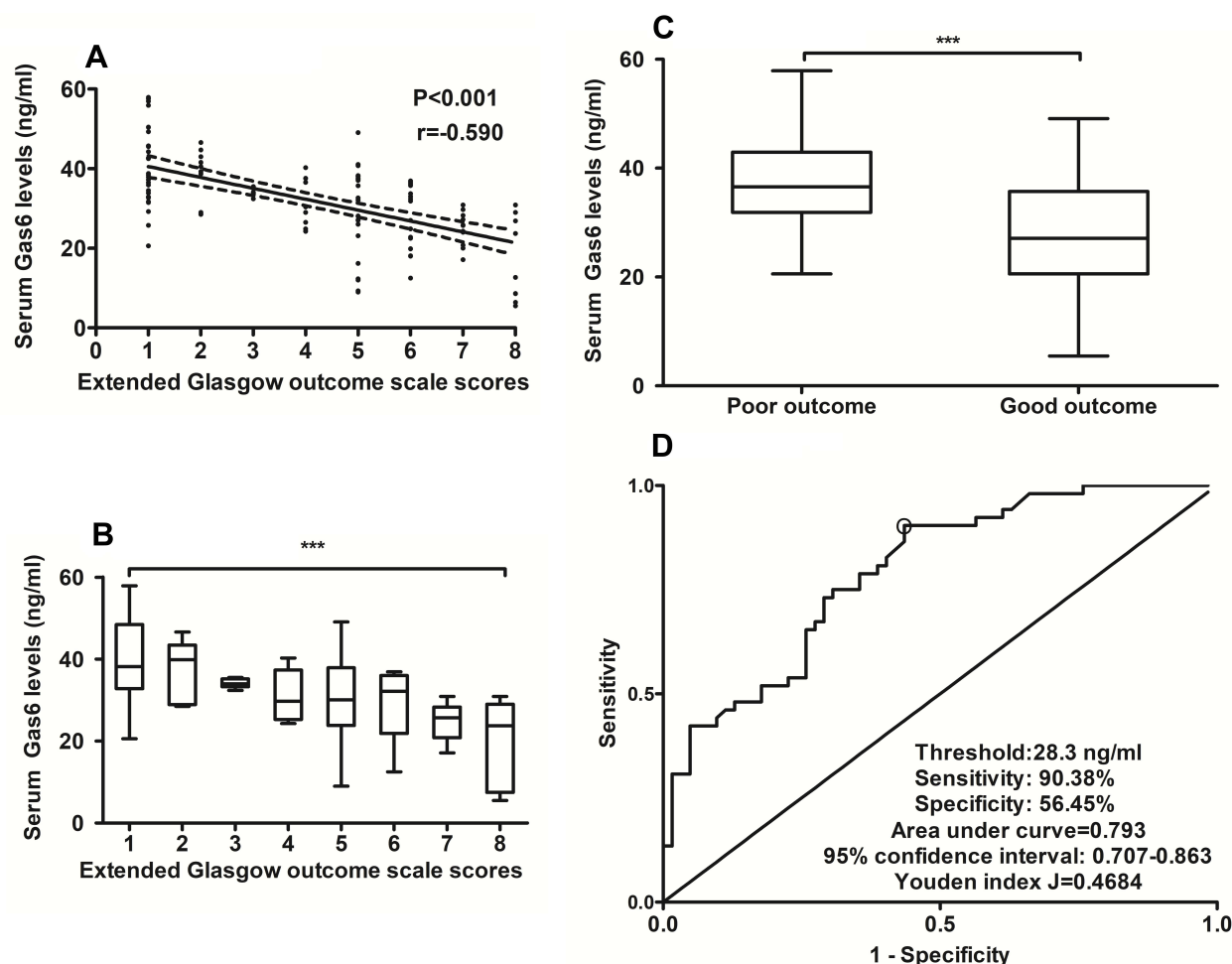
	Hazard Ratio	95% CI	P value
Gender (male/female)	1.041	0.488–2.223	0.917
Age (y)	1.010	0.984–1.038	0.453
Current cigarette smoking	1.441	0.675–3.078	0.345
Alcohol abuse	1.371	0.642–2.928	0.414
Hypertension	1.765	0.750–4.154	0.193
Diabetes mellitus	2.282	0.925–5.629	0.073
Hyperlipidemia	1.169	0.474–2.883	0.735
Admission time after trauma (h)	0.926	0.800–1.072	0.304
Time from trauma to sampling (h)	0.916	0.802–1.046	0.195
Traumatic causes			
Automobile/motorcycle	Reference		
Fall/jump	0.676	0.289–1.579	0.365
Others	1.513	0.506–4.529	0.459
Glasgow coma scale score	0.395	0.272–0.574	<0.001
Systolic arterial pressure (mmHg)	0.992	0.980–1.005	0.253
Diastolic arterial pressure (mmHg)	0.997	0.974–1.019	0.774
Rotterdam CT classification	3.731	2.343–5.942	<0.001
Abnormal cisterns	4.532	1.075–19.102	0.039
Midline shift > 5 mm	3.112	1.183–8.191	0.021
Epidural hematoma	2.343	1.032–5.320	0.042
Subdural hematoma	1.923	0.847–4.365	0.118
Subarachnoid hemorrhage	1.718	0.730–4.041	0.215
Intraventricular hemorrhage	2.041	0.775–5.371	0.149
Intracerebral hematoma	1.670	0.756–3.692	0.205
Brain contusion	1.217	0.562–2.637	0.618
Pneumocephalus	1.331	0.629–2.813	0.455
Blood glucose levels (mmol/l)	1.135	1.016–1.268	0.025
Blood leucocyte count ( $\times 10^9/l$ )	1.090	0.951–1.250	0.216
Serum Gas6 levels (ng/mL)	1.144	1.074–1.217	<0.001

**Note:** Results were generated using univariate Cox's proportional hazard regression analysis.

**Abbreviations:** CT, computerized tomography; Gas6, growth arrest-specific protein 6; 95% CI, 95% confidence interval.

In order to verify the relationship between serum Gas6 levels and traumatic severity plus prognosis, we configured the four multivariate models, namely, one linear regression model, two binary logistic regression models and one Cox's proportional hazard model. Interestingly, serum Gas6 levels were found to be independently correlated with GCS score and Rotterdam CT score, as well as to be independently associated with 180-day death, overall survival and poor outcome. Clearly, GCS and Rotterdam CT classification are the two common prognostic determinants of head trauma.<sup>3–5,30</sup> Thus, it is deduced that serum Gas6 levels may be tightly related to trauma severity and long-term clinical outcome.

Two limitations warrant to be mentioned in this study. First, although this is the first study to investigate the prognostic role of serum Gas6 levels in sTBI and further find some interesting results, this is only a preliminary study because of its relatively small sample size. Therefore, a large cohort study is needed to validate such conclusions. Second, GCS and Rotterdam CT classification currently serve as the two common prognostic predictors of TBI.<sup>3–5,30</sup> Some biomarkers, eg, S100B, glial fibrillary acidic protein, ubiquitin carboxyl-terminal hydrolase L1, myelin basic protein and neuron-specific enolase, are confirmed to be potential prognostic biomarkers of TBI.<sup>9–11</sup> The interesting finding in the current study is that serum Gas6 levels showed similar prognostic predictive ability, as



**Figure 5** Relationship between serum growth arrest-specific protein 6 levels and 180-day poor outcome in patients with severe traumatic brain injury. **(A)** Relationship between serum growth arrest-specific protein 6 levels and extended Glasgow coma scale scores in patients with severe traumatic brain injury. **(B)** Comparisons of serum growth arrest-specific protein 6 levels among subgroups with different extended Glasgow coma scale scores in patients with severe traumatic brain injury. **(C)** Comparisons of serum growth arrest-specific protein 6 levels between patients with good prognosis and those with poor prognosis. **(D)** Serum growth arrest-specific protein 6 levels with respect to predictive capability for 180-day poor prognosis. Whether extended Glasgow outcome scale score was considered as a continuous or categorical variable, their close correlations still existed (\*\* $P < 0.001$ ). As compared with patients with good prognosis (extended Glasgow outcome scale score 5–8), serum growth arrest-specific protein 6 levels were substantially increased in those with poor prognosis (extended Glasgow outcome scale score 1–4) (\*\* $P < 0.001$ ). Serum growth arrest-specific protein 6 levels significantly distinguished patients at risk of 180-day poor prognosis ( $P < 0.001$ ). Gas6 indicates growth arrest-specific protein 6.

compared to GCS score and Rotterdam CT score. Thus, our future mission is to choose a biomarker from the preceding biomarkers, including Gas6, which can efficiently improve the prognostic capability when it is combined with GCS score or Rotterdam CT score.

## Conclusions

This is the first series for investigating the relationship between serum Gas6 levels and trauma severity plus long-term clinical outcome of sTBI. The main findings are that elevated serum Gas6 levels after sTBI are independently correlated with clinical and radiological severity indicated by GCS score and Rotterdam CT classification, as well as independently predict long-term outcome, including mortality, overall survival and poor prognosis. Hence, serum Gas6 may serve as a useful prognostic biomarker for sTBI.

**Table 4** Factors Associated with 180-Day Poor Outcome After Severe Traumatic Brain Injury

	GOSE 1–4	GOSE 5–8	P value
Gender (male/female)	30/22	38/24	0.697
Age (y)	44.5±14.4	42.8±13.7	0.527
Current cigarette smoking	20 (38.5%)	15 (24.2%)	0.100
Alcohol abuse	17 (32.7%)	20 (32.3%)	0.961
Hypertension	11 (21.2%)	8 (12.9%)	0.239
Diabetes mellitus	10 (19.2%)	4 (6.5%)	0.038
Hyperlipidemia	11 (21.2%)	11 (17.7%)	0.646
Admission time after trauma (h)	4.6 (3.0–6.1)	4.8 (4.1–6.4)	0.157
Time from trauma to sampling (h)	6.2 (4.1–8.0)	6.5 (5.2–8.2)	0.169
Traumatic causes			0.157
Automobile/motorcycle	26	33	
Fall/jump	18	26	
Others	8	3	
Glasgow coma scale score	4 (3–5)	6 (5–7)	<0.001
Systolic arterial pressure (mmHg)	119.5±29.2	129.4±27.4	0.064
Diastolic arterial pressure (mmHg)	72.1±17.5	75.6±15.5	0.267
Rotterdam CT classification	5 (4–6)	4 (4–4)	<0.001
Abnormal cisterns	46 (88.5%)	41 (66.1%)	0.005
Midline shift > 5 mm	41 (78.9%)	30 (48.4%)	0.001
Epidural hematoma	35 (67.3%)	26 (41.9%)	0.007
Subdural hematoma	37 (71.2%)	29 (46.8%)	0.009
Subarachnoid hemorrhage	39 (75.0%)	35 (56.5%)	0.039
Intraventricular hemorrhage	7 (13.5%)	5 (8.1%)	0.350
Intracerebral hematoma	33 (63.5%)	32 (51.6%)	0.203
Brain contusion	36 (69.2%)	33 (53.2%)	0.082
Pneumocephalus	18 (34.6%)	25 (40.3%)	0.531
Blood glucose levels (mmol/l)	8.8 (7.0–11.9)	7.2 (5.6–9.5)	0.010
Blood leucocyte count ( $\times 10^9/l$ )	8.9 (6.5–11.3)	7.5 (5.8–9.6)	0.075
Serum Gas6 levels (ng/mL)	36.6 (32.1–42.9)	27.2 (20.8–35.7)	<0.001

**Notes:** Quantitative data were presented as mean  $\pm$  standard deviation if normally distributed and as median (25th–75th percentiles) if non-normally distributed. Qualitative data were reported as counts (percentages). Intergroup comparisons of data were done using the t-test, Mann–Whitney U-test, Pearson chi-square test or Fisher's exact test as appropriate.

**Abbreviations:** CT, computerized tomography; Gas6, growth arrest-specific protein 6; GOSE, Extended Glasgow outcome scale.

## Abbreviations

CT, computerized tomography; GCS, Glasgow coma scale; ROC, receiver operating characteristic; sTBI, severe traumatic brain injury; AUC, area under receiver operating characteristic curve; OR, odds ratio; HR, hazard ratio; VKDPs, vitamin K-dependent proteins; Gas6, growth arrest-specific protein 6; GOSE, extended Glasgow Outcome Scale.

## 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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