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Higher plasma C-type lectin-like receptor 2 concentrations for prediction of higher risk of 30-day mortality in isolated severe blunt traumatic brain injury

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ABSTRACT

Background: Platelet activation is implicated in secondary brain injury following traumatic brain injury (TBI). C-type lectin-like receptor 2 (CLEC-2) is extensively expressed on platelets and participates in platelet activation. We investigate dthe prognostic significance of plasma CLEC-2 in TBI patients.

Methods: One hundred and six patients with isolated severe blunt TBI and 106 healthy controls were prospectively investigated. Plasma CLEC-2 concentrations were detected and Glasgow coma scale (GCS) scores were recorded. The relationship between plasma CLEC-2 concentrations and 30-day mortality in addition to overall survival was determined using multivariate models.

Results: Patients exhibited a substantially higher concentration of plasma CLEC-2 than healthy controls. Among patients, plasma CLEC-2 concentrations were remarkably increased in the GCS scores- and Rotterdam computerized tomography classification- dependent manner. As compared with survivors within posttraumatic 30 days, plasma CLEC-2 concentrations were remarkably raised in non-survivors. Rising plasma CLEC-2 concentration was independently associated with an enhanced risk of 30-day mortality and short overall survival time. Plasma CLEC-2 concentrations had a significantly high area under receiver operating characteristic curve for predicting 30-day mortality.

Conclusions: Incremental plasma CLEC-2 concentrations are intimately related to increasing trauma severity, in close association with increased 30-day death, indicating the prognostic role of plasma CLEC-2 in TBI.

1. Introduction

Traumatic brain injury (TBI) is a major cause of global disease burden with high mortality and disability rate [1–3]. Primary brain injury and secondary brain injury are the two important pathophysiological mechanisms underlying traumatized brain injury. Primary brain injury is directly caused by external force and thereby, its prevention is impossible. However, secondary brain injury is related to inflammation, coagulation disturbances, apoptosis and other factors [4–6]. The therapies for such brain injury are necessary. Among TBI patients, cases with coagulation disturbances account for more than one third [7–13]. Reportedly, platelet dysfunction has played a crucial role in occurrence and development of coagulation disturbances [14–17]. The mechanisms that lead to platelet dysfunction in the acute response to TBI are poorly understood [18–22]. Admittedly, this dysfunction is associated with early platelet activation. Growing evidence shows that platelet activation is essentially involved in the progression of secondary brain injury after TBI, possibly by orchestrating a complex thromboinflammatory reaction in which interactions between platelets, immune cells, and the contact activation system disrupt the blood-brain barrier and damage neurological function [23–25]. CLEC-2 (C-type lectin receptor 2) is a member of the family of Ca2 + −dependent proteins with carbohydrate-binding ability, which is widely expressed on the plasma membranes of platelets and can activate platelets either by its endogenous ligand podoplanin or by the snake venom toxin rhodocytin [26–31]. CLEC-2 is an essential platelet-activating receptor in hemostasis and thrombosis [32]. It promotes hematogenous tumor metastasis and prothrombotic state in tumor-bearing mice [33]. Interestingly, CLEC-2 has been found to play an essential role in formation of atherosclerotic plaques [34]. Also, increased plasma CLEC-2 concentrations were highly associated with stroke progression and poor prognosis at 90 days after acute ischemic stroke as well as 1-y death and

Abbreviations: CT, computerized tomography; GCS, Glasgow coma scale; TBI, traumatic brain injury; CLEC-2, C-type lectin-like receptor 2

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vascular diseases in patients with acute ischemic stroke [35,36]. Up to now, circulating CLEC-2 concentrations have not yet been measured in patients with TBI. This study was designed to ascertain the relationship between plasma CLEC-2 concentrations and prognosis of TBI patients.

2. Materials and methods

2.1. Subjects

Subjects were recruited with the written informed consents from their relatives after the study protocol was approved by the Ethics Committee at the Shengzhou People's Hospital. In this prospective and observational study performed at the Shengzhou People's Hospital between January 2015 and May 2018, we consecutively enrolled isolated severe blunt TBI adults (age of ≥18 y) admitted within 12 h following head trauma. Trauma severity was assessed using Glasgow Coma Scale (GCS). Severe TBI was defined as GCS score < 9 while patients were not under the influence of pharmacologic agents or alcohol. Isolated TBI was considered as injury severity score in non-cranial aspects < 9 points. Patients with autoimmune diseases, severe infection, severe hepatic, renal, cardiac or respiratory diseases, pregnancy, and known malignancy were excluded. In the same period, we enrolled healthy individuals as controls.

2.2. Data collection and outcome measure

Some information, such as demographics, comorbidities and time from trauma to hospitalization, were collected upon arrival at emergency room. Traumatic causes were classified to automobile/motorcycle, fall/jump and others. We observed pupillary reactivity of each patient. Trauma severity was assessed using GCS score. An initial head computerized tomography (CT) scan at admission and at least a follow-up CT scan within 6 h after admission (expect those undergoing an immediate operation) were done. The positive trauma-related cerebral lesions confirmed by head computerized tomography scan included skull-cap fracture, skull-base fracture, epidural hematoma, subdural hematoma, subarachnoid hemorrhage, intraventricular hemorrhage, cerebral hematoma, brain contusion and pneumocephalus. Both progressive hemorrhagic brain injury and posttraumatic cerebral infarction were read. Other radiological appearances, e.g. abnormal cisterns and midline shift, were also recorded. Brain lesion was outlined in accordance with the Rotterdam CT classification. The clinical outcome was 30-day mortality.

2.3. Immune analysis

Venous blood samples were collected in tubes containing ethylenediaminetetraacetic acid as an anticoagulant agent. Plasma was acquired by centrifugation for 10 min at 3000 rpm and preserved at −80 °C until assayed. Plasma CLEC-2 concentrations were quantified in duplicate using enzyme-linked immunosorbent assay kits following the manufacturer's instructions (Xinle, Shanghai, China).

2.4. Statistical analysis

Baseline characteristics were summarized as median (interquartile range) for continuous variables and as number (%) for categorical variables. Comparison was carried out using a Kruskal-Wallis test, Wilcoxon-Mann-Whitney test, Fisher's exact test or x^2 test where appropriate. Bivariate correlations were analyzed by Spearman correlation coefficients. Baseline characteristics between the 2 groups (non-survivors and survivors) were compared, and the factors with $P < .05$ were incorporated in the binary logistic regression model. Odds ratio and 95% confidence interval were calculated. The Kaplan–Meier method was utilized to estimate 30-day overall survival time. Survival time was compared with the log-rank test. The univariate Cox's proportional hazard analysis was done to ascertain factors associated with 30-day overall survival time. The factors with $P < .05$ were included in the multivariate Cox's proportional hazard model. Hazard ratio (HR) and 95% CI were estimated. Receiver operating characteristic curve of plasma CLEC-2 concentrations for prediction of 30-day death was drawn. The predictive ability was reported as area under curve (AUC) and 95% CI. All statistical analyses were 2-tailed. A $P < .05$ was considered statistically significant. SPSS, ver 18.0 (IBM) was utilized for all statistical analyses.

3. Results

3.1. Subjects

During the study period, we recruited a total of 142 adult patients with isolated severe blunt TBI admitted within post-traumatic 12 h according to selection criteria. Afterwards, in accordance to exclusion criteria, we removed 36 subjects. At last, 106 cases were available for analysis. Also, a total of 106 healthy controls were enrolled. By statistical analysis, there were not statistical significant differences for age and gender proportion between 106 included patients and 106 included controls as well as between 106 included and 36 excluded patients.

Among those included patients, of whom males accounted for 60.4%, age ranged from 18 to 74 y (median, 43 y; the upper - lower quartiles, 32–53 y). Comorbidities included hyperlipidemia in 19 patients, hypertension in 14 patients and diabetes mellitus in 13 patients. Among cases, smokers constituted 46.2% and alcohol drinkers accounted for 52.8%. Traumatic causes contained automobile/motorcycle in 53 patients, fall/jump in 40 patients and others in 13 patients. Patients were admitted from 0.5 to 12 h after head trauma, with a median time of 4.2 h (the upper - lower quartiles, 3.4–5.3 h). Among those 106 patients, 14 patients had GCS score 3; 31 patients, GCS score 4; 22 patients, GCS score 5; 13 patients, GCS score 6; 13 patients, GCS score 7 and 13 patients, GCS score 8. In total, 50 patients bore pupil dilation The trauma-related radiological appearances were skull-cap fracture (70 patients), skull-base fracture (56 patients), epidural hematoma (50 patients), subdural hematoma (70 patients), subarachnoid hemorrhage (75 patients), intraventricular hemorrhage (11 patients), intracerebral hematoma (63 patients), brain contusion (70 patients), pneumocephalus (34 patients), midline shift > 5 mm (68 patients), posttraumatic cerebral infarction (14 patients), progressive hemorrhagic brain injury (29 patients) and abnormal basal cisterns (84 patients). Via the initial CT scans, we found that 14, 26, 28 and 38 patients presented with the Rotterdam CT classification 3, 4, 5 and 6 respectively. Venous blood was acquired from 2.2 to 15.4 h following trauma (median, 6.3 h; the upper - lower quartiles, 5.5–7.3 h). The 30-day mortality was 26.4% (28/106) and the 30-day mean overall survival time was 24.5 d (95% CI, 22.7–26.4 d).

3.2. Change in plasma CLEC-2 concentrations and association with severity

As shown in Fig. 1, patients had significantly increased median concentrations of plasma CLEC-2, in comparison with controls; as opposed to survivors, non-survivors exhibited substantially raised median concentrations of plasma CLEC-2; also, median plasma CLEC-2 concentrations in survivors were remarkably higher than those in controls. In this study, we used the GCS scores and the Rotterdam CT classification to assess the trauma severity. It was revealed that, with incremental severity indicated by both decreasing GCS scores and increasing Rotterdam CT classification, plasma CLEC-2 concentrations were substantially raised whether by bivariate intergroup comparisons or bivariate correlation analyses (Figs. 2-5).

3.3. Mortality analysis

Under ROC curve, plasma CLEC-2 concentrations were at state of a

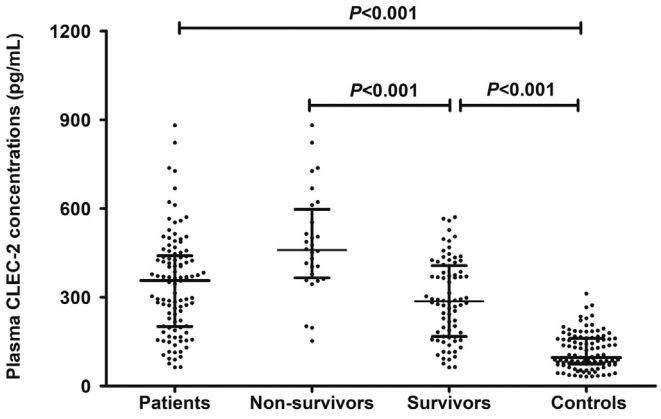

Fig. 1. Differences of plasma C-type lectin - like receptor 2 concentrations between healthy controls and head-traumatized patients as well as between non-survivors and survivors within 30 days following traumatic brain injury. CLEC-2 denotes C-type lectin - like receptor 2.

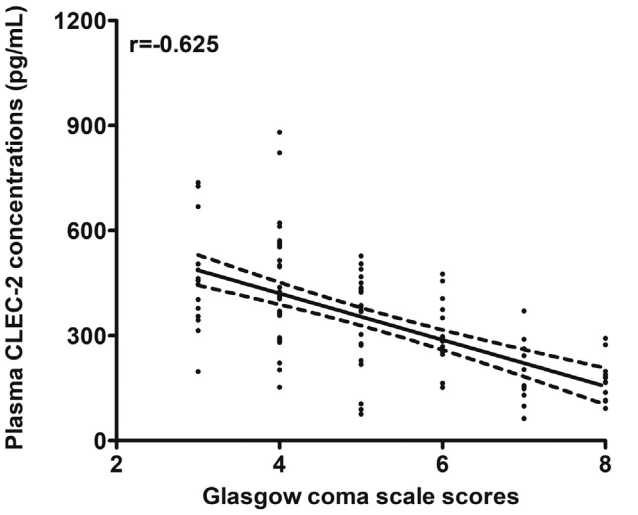

Fig. 2. Correlative curve between Glasgow coma scale scores and plasma C-type lectin - like receptor 2 concentrations. CLEC-2 denotes C-type lectin - like receptor 2.

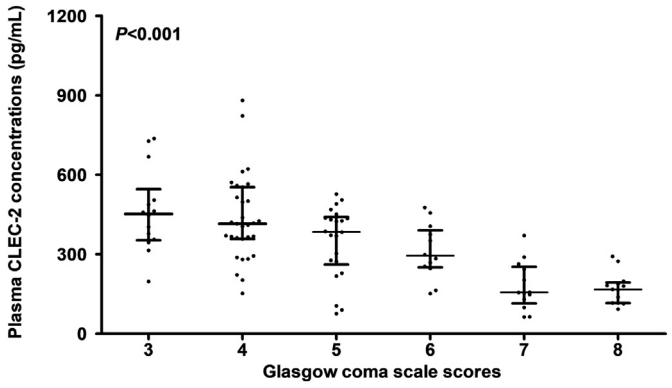

Fig. 3. Differences of plasma C-type lectin - like receptor 2 concentrations by Glasgow coma scale scores. CLEC-2 denotes C-type lectin - like receptor 2.

remarkably high discriminatory capability for 30-day death. Moreover, in Fig. 6, plasma CLEC-2 concentrations > 314 pg/ml distinguished the patients at risk of 30-day death with the medium-high sensitivity and specificity values. Additionally, patients with plasma CLEC-2 concentrations > 314 pg/ml had the markedly shorter overall survival time

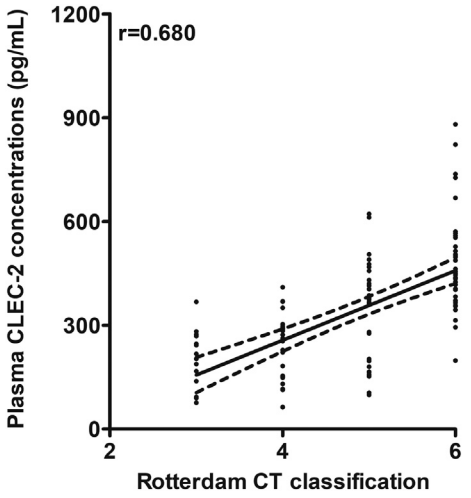

Fig. 4. Correlative curve between Rotterdam computerized tomography classification and plasma C-type lectin - like receptor 2 concentrations. CLEC-2 denotes C-type lectin - like receptor 2. CT means computerized tomography.

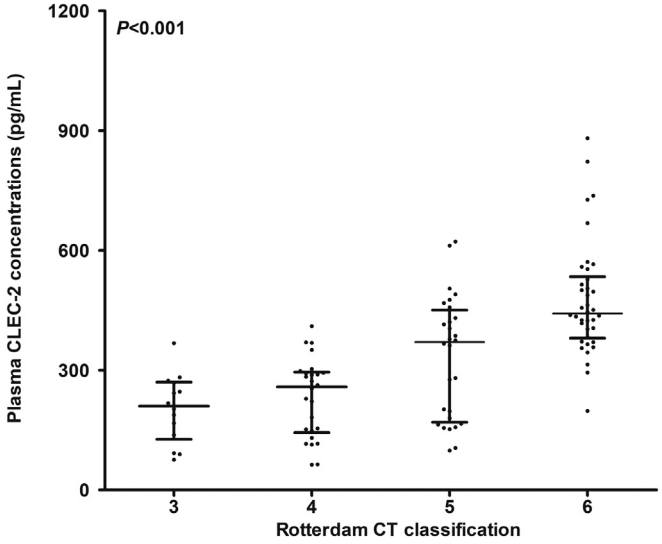

Fig. 5. Differences of plasma C-type lectin - like receptor 2 concentrations by Rotterdam computerized tomography classification. CLEC-2 denotes C-type lectin - like receptor 2. CT means computerized tomography.

than the remainders (Fig. 7).

In the current study, we utilized a binary logistic regression model and a multivariate Cox's proportional hazard model respectively to discern whether plasma CLEC-2 concentrations were independently associated with 30-day mortality and 30-day overall survival. Univariate analyses showed that 30-day mortality and overall survival might be relate to GCS scores, Rotterdam CT classification, plasma CLEC-2 concentrations > 314 pg/ml and other variables listed in Tables 1 and 2. Furthermore, when the preceding significant variables were incorporated into the multivariate models, we found that plasma CLEC-2 concentrations > 314 pg/ml (OR = 4.28, 95% CI = 1.03–17.78, $P = .025$), Rotterdam CT classification (OR = 2.05, 95% CI = 1.02–4.12, $P = .033$) and GCS score (OR = 0.31, 95% CI = 0.16–0.61, $P = .001$) remained independently associated with 30-day mortality after head trauma, as well as they predicted 30-day overall survival with HR values of 3.67, (95% CI = 1.09–12.34, $P = .016$), 1.77 (95% CI = 1.02–3.08, $P = .032$) and 0.35 (95% CI = 0.22–0.57, $P < .001$) respectively.

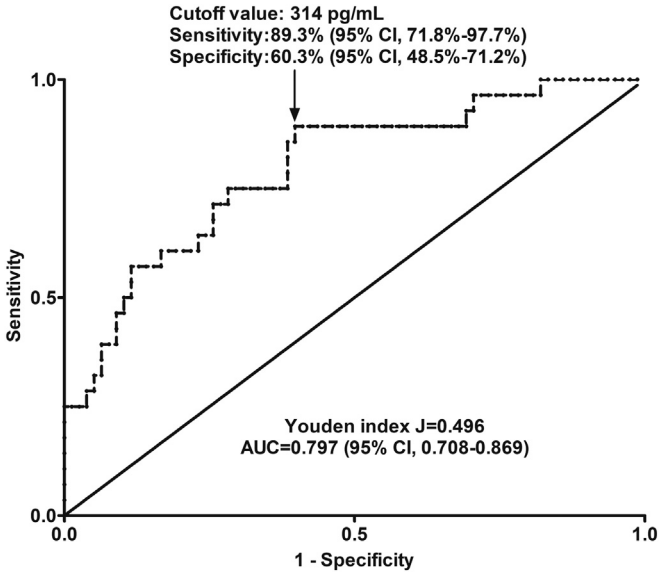

Fig. 6. Receiver operating characteristic curve of plasma C-type lectin-like receptor 2 concentrations for prediction of death within thirty days after traumatic brain injury. AUC indicates area under curve and 95% CI means 95% confidence interval.

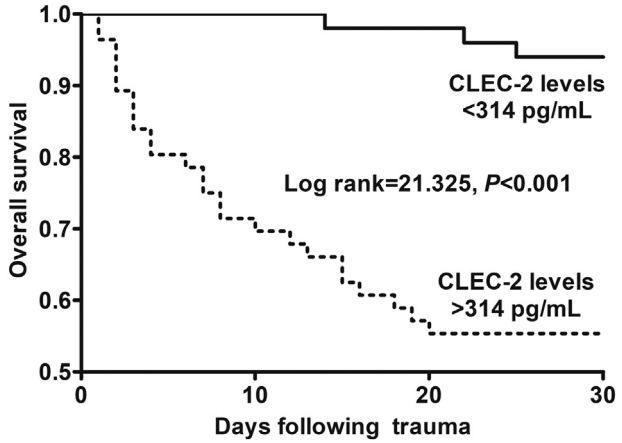

Fig. 7. Survival curve showing significant difference in posttraumatic 30-day overall survival by plasma C-type lectin-like receptor 2 concentrations. CLEC-2 denotes C-type lectin - like receptor 2.

4. Discussion

Based on our findings, it is presumed that elevation of plasma CLEC-2 concentrations was independently associated with poor prognosis in a group of Chinese TBI patients. First, plasma CLEC-2 concentrations were substantially higher in TBI patients than in healthy individuals. Interestingly, among the patients, escalating CLEC-2 concentrations were correlated with both declining GCS scores and rising Rotterdam CT classification. Second, patients with high plasma CLEC-2 concentrations (> 314 pg/ml) were 3- to 4-fold more likely to have 30-day death risk, compared with the low concentrations (< 314 pg/ml). Also, plasma CLEC-2 could provide independent predictive information for 30-day mortality. Results were further confirmed in the multivariate analysis with plasma CLEC-2 as a categorical variable. In addition, we revealed that plasma CLEC-2 was an independent predictor for 30-day overall survival time. Moreover, this biomarker performed moderately well in outcome prediction for 30-day mortality under the ROC curve. The above-mentioned data indicate that plasma CLEC-2 might be used as a valuable biomarker for assessment of severity and prognostication.

Table 1
The parameters related to 30-day mortality following traumatic brain injury

	The deceased (n = 28)	The alive (n = 78)	*P* value
Gender (male/female)	17/11	47/31	NS
Age (y)	52 (35-60)	40 (30-51)	0.013
Hyperlipidemia	6 (21.4%)	13 (16.7%)	NS
Hypertension	6 (21.4%)	8 (10.3%)	NS
Diabetes mellitus	5 (17.9%)	8 (10.3%)	NS
Smokers	13 (46.4%)	36 (46.2%)	NS
Alcohol use	11 (39.3%)	45 (57.7%)	NS
Traumatic causes			NS
Automobile/motorcycle	16	37	
Fall/jump	6	34	
Others	6	7	
Glasgow Coma Scale scores	4 (3-4)	5 (4-7)	< 0.001
Pupil dilation	26 (92.9%)	24 (30.8%)	< 0.001
The trauma-related radiological appearances			
Skull-cap fracture	20 (71.4%)	50 (64.1%)	NS
Skull-base fracture	19 (67.9%)	37 (47.4%)	NS
Epidural hematoma	19 (67.9%)	31 (39.7%)	0.011
Subdural hematoma	20 (71.4%)	50 (64.1%)	NS
Subarachnoid hemorrhage	24 (85.7%)	51 (65.4%)	0.042
Intraventricular hemorrhage	8 (28.6%)	3 (3.9%)	< 0.001
Intracerebral hematoma	17 (60.7%)	46 (59.0%)	NS
Brain contusion	21 (75.0%)	49 (62.8%)	NS
Pneumocephalus	8 (28.6%)	26 (33.3%)	NS
Posttraumatic cerebral infarction	10 (35.7%)	4 (5.1%)	< 0.001
Progressive hemorrhagic brain injury	13 (46.2%)	16 (20.5%)	0.008
Abnormal cisterns	26 (92.9%)	58 (74.4%)	0.038
Midline shift > 5 mm	24 (85.7%)	44 (56.4%)	0.006
Rotterdam CT classification	6 (5-6)	4 (4-6)	< 0.001
Time from trauma to hospitalization (h)	4.3 (3.5-5.7)	4.2 (3.4-5.5)	NS
Time from trauma to blood-collection (h)	6.3 (6.1-7.3)	6.2 (4.8-7.2)	NS
Systolic arterial pressure (mmHg)	121 (91-141)	127 (98-140)	NS
Diastolic arterial pressure (mmHg)	76 (60-98)	74 (58-92)	NS
Blood glucose levels (mmol/l)	13.1 (10.4-18.3)	11.4 (9.1-12.9)	0.016
Serum C-reactive protein levels (mg/l)	18.1 (13.4-22.1)	14.4 (12.7-17.4)	0.015
Blood white blood cell count ($\times 10^9$/l)	10.6 (5.7-13.8)	8.5 (6.9-9.7)	NS
Plasma CLEC-2 levels > 314 pg/ml	25 (89.3%)	31 (39.7%)	< 0.001

CT indicates computerized tomography and CLEC-2 denotes C-type lectin-like receptor 2. Baseline characteristics were summarized as median (interquartile range) for continuous variables and as number (percent) for categorical variables. Comparison was done using the Wilcoxon-Mann-Whitney test, Fisher's exact test or χ^2 test as appropriate.

Acute traumatic coagulopathy is prevalent in cases with severe TBI and its incidence exceeds 60% [7–13]. It has been evidenced that acute traumatic coagulopathy is an independent prognostic risk factor after head trauma and increases the chances of poor outcome by > 30-fold relative to patients without coagulopathy [7–13]. The underlying mechanisms for the TBI-induced coagulopathy are not completely clear but may involve platelet activation, subsequently leading to platelet dysfunction [23–25]. CLEC-2 is a one of (hem) immunoreceptor tyrosine-based activation motif-containing receptors on platelets, eliciting powerful platelet activation signals in conjunction with Syk kinases [26–31]. Evidence has shown CLEC-2 has a synergistic effect on glycoprotein VI in hemostasis and arterial thrombosis [37]. In our present study, an elevation of plasma CLEC-2 concentrations in TBI patients, in comparison with healthy controls, had been found. Thus, it is assumed that CLEC-2 might play an important role in TBI-elicited brain injury. However, more studies, especially animal experiments, are warranted to disentangle the causal relationship and underlined mechanisms.

Table 2
The components associated with 30-overall survival after head trauma

	Hazard ratio	95% confidence interval	*P* value
Gender (male/female)	1.015	0.475~2.167	NS
Age (y)	1.035	1.008~1.062	0.011
Hyperlipidemia	1.269	0.515-3.131	NS
Hypertension	2.030	0.823-5.010	NS
Diabetes mellitus	1.758	0.668-4.626	NS
Smokers	1.006	0.478-2.113	NS
Alcohol use	0.509	0.238-1.087	NS
Traumatic causes			
Automobile/motorcycle	Reference		
Fall/jump	0.497	0. 194-1.270	NS
Others	1.644	0.642-4.208	NS
Glasgow Coma Scale scores	0.319	0.202~0.502	< 0.001
Pupil dilation	19.437	4.605-82.045	< 0.001
The trauma-related radiological appearances			
Skull-cap fracture	1.344	0.592-3.051	NS
Skull-base fracture	2.065	0.934-4.656	NS
Epidural hematoma	2.684	1.214-5.935	0.015
Subdural hematoma	1.332	0.587-3.025	NS
Subarachnoid hemorrhage	2.751	1.054-7.930	0.048
Intraventricular hemorrhage	4.689	2.057-10.685	< 0.001
Intracerebral hematoma	1.045	0.490-2.233	NS
Brain contusion	1.622	0.690-3.817	NS
Pneumocephalus	0.858	0.378-1.948	NS
Posttraumatic cerebral infarction	5.563	2.548-12.146	< 0.001
Progressive hemorrhagic brain injury	2.681	1.274-5.640	0.009
Abnormal cisterns	3.827	1.098~16.126	0.047
Midline shift > 5 mm	3.849	1.335-11.101	0.013
Rotterdam CT classification	2.715	1.622-4.547	< 0.001
Time from trauma to hospitalization (h)	0.994	0.853-1.158	NS
Time from trauma to blood-collection (h)	0.995	0.864-1.147	NS
Systolic arterial pressure (mmHg)	0.997	0.984-1.010	NS
Diastolic arterial pressure (mmHg)	1.004	0.985-1.023	NS
Blood glucose levels (mmol/l)	1.147	1.046-1.258	0.003
Serum C-reactive protein levels (mg/l)	1.109	1.025-1.200	0.010
Blood white blood cell count ($\times 10^9$/l)	1.262	1.095-1.454	0.001
Plasma CLEC-2 levels > 314 pg/ml	9.851	2.969-32.688	< 0.001

CT and CLEC-2 indicates computerized tomography and C-type lectin-like receptor 2 respectively. Associations with overall survival were reported as hazard ratio values using the univariate Cox's proportional hazard analysis.

A more recent study [35] showed that plasma CLEC-2 concentrations were predominantly raised in patients with acute ischemic stroke than in healthy controls, which is similar to the results in our study. The authors investigated stroke progression (defined as any new neurological symptoms/signs or any neurological worsening within 7 days after stroke onset) and poor prognosis (defined as modified Rankin scale scores > 2 at 90 days), and further found that escalating plasma CLEC-2 concentrations were independently associated with stroke progression and poor prognosis at 90 days significantly. Another study identified plasma CLEC-2 as a predictor of 1-y death and vascular events in patients with acute ischemic stroke [36]. Such the two studies indicate that plasma CLEC-2 might be a potential prognostic biomarker for acute ischemic stroke. However, this sort of epidemiological investigation has not been done in head trauma. Herein, we regarded 30-day death as a prognostic parameter, which is different from the above-mentioned study investigating long-term functional outcome. Our results might be astonishing because we not only revealed a close correlation of plasma CLEC-2 concentrations with trauma severity indicated by Glasgow coma scale score and Rotterdam CT classification, but also ascertained an independent relationship between plasma CLEC-2 concentrations and the short-term death, namely 30-day mortality and overall survival. More interestingly, there was a high prognostic predictive ability for plasma CLEC-2 concentrations to discriminate the patients at risk of death within 30 days following TBI. Taken together, it is implied that plasma CLEC-2 could represent a promising prognostic biomarker for head trauma.

5. Conclusions

We confirmed the role of ascending plasma CLEC-2 in assessment of short-term death and trauma severity after TBI, hinting plasma CLEC-2 could be a new biomarker for prognosis of head trauma.

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