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Low serum calcium is a novel predictor of unfavorable prognosis after traumatic brain injury

Tian Li [a], Dongzhou Zhuang [b,e], Shirong Cai [b], Faxiu Ding [b], Fei Tian [c], Mindong Huang [d], Lianjie Li [e], Weiqiang Chen [b,**], Kangsheng Li [a,*], Jiangtao Sheng [a,***]

[a] *Shantou University Medical College, Department of Microbiology and Immunology & Guangdong Provincial Key Laboratory of Infectious Diseases and Molecular Immunopathology, Shantou, Guangdong, China*
[b] *First Affiliated Hospital of Shantou University Medical College, Department of Neurosurgery, Shantou, Guangdong, China*
[c] *Second Affiliated Hospital of Shantou University Medical College, Department of Neurosurgery, Shantou, Guangdong, China*
[d] *Affiliated Jieyang Hospital of Sun Yat-sen University, Department of Neurosurgery, Jieyang, Guangdong, China*
[e] *Fuzong Clinical Medical College of Fujian Medical University, Department of Neurosurgery, Fuzhou, Fujian, China*

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ABSTRACT

Background: Accurate and convenient serological markers for prognosis after traumatic brain injury (TBI) are still lacking. We aimed to explore the predictive value of serum calcium for prognosing outcomes within 6 months after TBI.

Methods: In this multicenter retrospective study, 1255 and 719 patients were included in development and validation cohorts, respectively, and their 6-month prognoses were recorded. Serum calcium was measured through routine blood tests within 24 h of hospital admission. Two multivariate predictive models with or without serum calcium for prognosis were developed. Receiver operating characteristics and calibration curves were applied to estimate their performance.

Results: The patients with lower serum calcium levels had a higher frequency of unfavorable 6-month prognosis than those without. Lower serum calcium level at admission was associated with an unfavorable 6-month prognosis in a wide spectrum of patients with TBI. Lower serum calcium level and our prognostic model including calcium performed well in predicting the 6-month unfavorable outcome. The calcium nomogram maintained excellent performance in discrimination and calibration in the external validation cohort.

Conclusions: Lower serum calcium level upon admission is an independent risk factor for an unfavorable 6-month prognosis after TBI. Integrating serum calcium into a multivariate predictive model improves the performance for predicting 6-month unfavorable outcomes.

Abbreviations: TBI, Traumatic brain injury; SDH, Subdural hematoma; SAH, Subarachnoid hemorrhage; GCS, Glasgow Coma Scale; CT, Computed tomography; EDH, Epidural hematoma; SICH, Spontaneous intracerebral hemorrhage; MAP, Mean arterial pressure; GOS, Glasgow Outcome Score; RBG, Random blood glucose; PT, Prothrombin time; APTT, Activated partial thromboplastin time; INR, International normalized ratio; ROC, Receiver operating characteristic; AUC, Area under receiver operating characteristic curve; OR, Odds ratio; CI, confidence interval.

* Corresponding author. Shantou University Medical College, 22 Xinling Road, Shantou, Guangdong, China.
** Corresponding author. First Affiliated Hospital, Shantou University Medical College, 57 Changping Road, Shantou, Guangdong , China
*** Corresponding author. Shantou University Medical College, 22 Xinling Road, Shantou, Guangdong, China.
E-mail addresses: wqchen@stu.edu.cn (W. Chen), ksli2013@yeah.net (K. Li), jtsheng@stu.edu.cn (J. Sheng).

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1. Introduction

Traumatic brain injury (TBI) is a leading cause of death and disability worldwide, and a major social, economic, and health problem [1,2]. Its progression is highly heterogeneous and profoundly affects patients from cognitive, somatic, emotional, and behavioral standpoints [2]. The heterogeneity makes prognosis after primary TBI difficult to predict.

In recent decades, studies on TBI prognosis have been carried out extensively. Many clinical factors, such as age, subdural hematoma (SDH), subarachnoid hemorrhage (SAH), coagulopathy, Glasgow Coma Scale (GCS) score, and oxygen saturation, have been reported to predict TBI prognosis [3]. However, the accuracy of these clinical factors for predicting TBI prognosis is limited. Some serum biomarkers, including S100B protein, glial fibrillar protein, ubiquitin C-terminal hydrolase-L1, and tau protein, have also been shown to have predictive value for TBI prognosis [4]. However, in actual clinical practice, these serum indicators are not routinely tested in patients with acute TBI and require further validation, limiting their practical value in predicting TBI prognosis.

Serological ion markers (represented by magnesium ion and phosphonium ion) have shown potential clinical diagnostic and prognostic value in patients with TBI [5]. Another divalent serum cation, calcium ion, has been shown to be involved in acute parenchymal hematoma progression after TBI or hemorrhagic stroke, potentially by affecting coagulation [6,7]. Notably, intracranial hemorrhage is a common and serious consequence after TBI, and its hematoma progression is associated with neurological deterioration, which in turn leads to poor prognosis in TBI patients [8,9]. Different from other divalent cations, calcium ions serve as intracellular second messengers that mediate multiple pathological processes, including neuronal degeneration [10,11]. Abnormal serum calcium level may reflect calcium homeostasis imbalance in the CNS, contributing to acute adverse progression and even leading to unfavorable long-term prognosis in patients after TBI.

As an objective indicator, serum calcium level can be quickly measured after admission and is an established valuable prognostic marker in stroke [6,12,13]. To date, the relationship between serum calcium and prognosis after TBI is unknown. Here, we aim to 1) investigate the association of hospital admission serum calcium level with unfavorable prognosis after primary TBI, and 2) develop and validate a useful clinical predictive tool for a 6-month unfavorable prognosis, following TBI, based on serum calcium level upon admission.

2. Methods

2.1. Study design and patients

This multicenter retrospective cohort study for establishing a multivariable predictive model of unfavorable prognosis after primary TBI was conducted in four triple-A teaching hospitals from March 2014 to October 2020. Between March 1, 2014, and February 1, 2019, consecutive patients with primary TBI at three Class A teaching hospitals (the Second Affiliated Hospital of Shantou University Medical College, Affiliated Jieyang Hospital of Sun Yat-sen University, and Affiliated East Hospital of Xiamen University Medical College, China) were retrospectively included in the development cohort. Between March 1, 2014, and October 1, 2020, consecutive patients with primary TBI at the First Affiliated Hospital of Shantou University Medical College were retrospectively included in the external validation cohort.

This study was approved by the ethics committees of the four hospitals mentioned above with ethical approval number (No. 2020-042). Given the anonymity of the data from the retrospective cohorts, the requirement for informed consent was waived. All procedures in this study followed the ethical standards and regulations in the appropriate version of the Declaration of Helsinki (World Medical Association, 2013) [14].

2.2. Inclusion and exclusion criteria

Inclusion criteria were as follows: (1) baseline computed tomography (CT) scans showing at least one intracranial hemorrhage, such as epidural hematoma (EDH), SDH, SAH, contusion, and intraventricular hemorrhage (IVH); and (2) hospital admission within 24 h of TBI. Exclusion criteria were as follows: (1) less than 18 years of age; (2) history of brain trauma or spontaneous intracerebral hemorrhage (SICH); (3) no initial blood test within 24 h after TBI; (4) massive blood transfusion; (5) presence of hepatic cirrhosis, hepatic dysfunction, kidney dysfunction, hyperthyroidism, hypothyroidism, chronic pancreatitis, any kind of tumor, and uncontrolled infection; (6) ingested anticoagulant or antiplatelet drugs before brain trauma; and (7) loss of 6-month prognostic information (Fig. S1).

2.3. Demographic and clinical variable assessment

Variables were obtained from the hospital's electronic medical record systems, including gender, age, hypertension, diabetes, smoking, neurosurgical intervention, GCS, admission mean arterial pressure (MAP), and in-hospital death. Patient long-term (6-month) prognosis was measured using a Glasgow Outcome Score (GOS) obtained through outpatient follow-up or telephone contact with patients 6 months after TBI. Venipuncture was performed on admission to collect venous blood samples. Routine blood tests and biochemical tests were conducted to determine platelet count, serum calcium levels, and random blood glucose (RBG). Routine coagulation tests were also performed on admission to determine the prothrombin time (PT), activated partial thromboplastin time (APTT), international normalized ratio (INR), and fibrinogen level.

2.4. Imaging acquisition and processing

Axial non-contrast CT images were accessed following a standardized local protocol at each participant's institution. Baseline CT images were reviewed by two readers (S.C. and D.Z.) blinded to the patient's clinical data. The presence of ICH, EDH, SDH, SAH, and IVH on the baseline and follow-up CT images (admission CT time <6 h after TBI) was analyzed with semi-automated computer-assisted volumetric analysis software (General Electric Company, Waukesha, WI, USA) [15]. The volumes of the hematomas were not calculated.

2.5. Definitions in this study

GCS scores were classified into three levels: mild (GCS score = 13–15), moderate (GCS score = 9–12), and severe (GCS score = 3–8) TBI [16]. Hypocalcemia was a serum calcium level below 2.10 mM/L [17]. Patient 6-month outcomes were divided into two groups according to the GOS score: unfavorable (GOS = 1–3) and acceptable (GOS = 4–5) [18]. Neurosurgical interventions included craniotomy for clot evacuation, decompressive craniectomy, ventriculoperitoneal shunt placement, extraventricular drainage, and other neurosurgeries. Injury mechanisms were divided a priori into three groups: severe, moderate, and mild [19]. To better represent the odds ratio of serum calcium level to the 6-month prognosis, the ratio of serum calcium level to the standard deviation of total group calcium level (Ca/CaSD) was used for the logistics regression analysis.

2.6. Statistical analyses

All statistical analyses were performed with the commercially available SPSS software version 26 (IBM Corporation, Armonk, New York, USA) and R statistical software (version 4.1.0, R Foundation, Vienna, Austria) with appropriate R packages [20]. Variables were compared in patients with and without hypocalcemia. Continuous variables are represented by the median (interquartile range), and categorical variables are represented by numerical values (percentages). The chi-square test was applied to categorical data, the Student's t-test to normally distributed variables, and the Mann-Whitney *U* test to nonparametric data. Differences in the area under the receiver operating characteristic curves (AUC), between models, were determined using the DeLong test. All tests of significance in this study were two-tailed, and a $P < 0.05$ was considered to indicate statistical significance. The Hosmer–Lemeshow test was conducted to assess the nomogram calibration. The Strengthening the Reporting of Observational Studies in Epidemiology and Transparent Reporting of an Individual Prognosis or Diagnosis Prediction model was used to prepare this manuscript[21,22].

2.6.1. Box plotting and two-stage linear regression

Box plotting and two-stage linear regression modeling were applied to observe the relationship between admission serum calcium level and 6-month prognosis. The inflection point of the smoothing function was calculated by a recursive algorithm. According to the inflection points, the odds ratios of serum calcium in both prognoses were assessed through univariate and multivariate regression analyses.

2.6.2. Subgroup analysis and interaction tests

The potential heterogeneity of serum calcium on unfavorable prognosis was further assessed by conducting subgroup analyses and interaction tests in the validation cohort.

2.6.3. Predictive factor selection

Predictive factor selection was first assessed by univariate logistic regression analysis and then a multivariate logistic regression analysis. In univariate regression analyses, variables with $P < 0.20$ were included in the multivariate analysis. Then these discriminative features were included in a multivariate logistic regression to construct a clinical model.

2.6.4. Construction of multivariate predictive models

After multivariate logistic regression, the following two predictive models for a 6-month prognosis were developed. The basic model included all independent risk factors selected by multivariate logistic regression except serum calcium, and the calcium model included serum calcium in addition to the variables in the basic model.

2.6.5. Model evaluation

The performance of the models was evaluated using receiver operating characteristic (ROC) curves and calibration curves. AUC was employed to assess the discriminatory ability of the predictive models. Calibration curves were plotted with the actual versus predicted probabilities for the assessment of the calibration of the model using 1000 bootstrap samples to reduce overfitting bias.

2.6.6. Nomogram construction

In the external validation cohort, a calcium nomogram was generated from the calcium model. Each regression coefficient in the multivariate regression was scaled and converted to a score of 0–100. The variable with the highest beta coefficient (absolute value) was assigned an effect score of 100. For the clinical application of the nomogram, the variables were carefully included, and the scores of each independent variable were summed to produce a total score, which was then converted into a predicted probability.

3. Results

3.1. Characteristics of patients with TBI according to hypocalcemia

The development cohort included 1255 patients with TBI (Fig. S1) and had a median serum calcium concentration of 2.26 (2.15–2.33) mM/L. Within the cohort, 106 (8.45%) patients died in the hospital and 270 (21.5%) had an unfavorable 6-month prognosis. The patients with hypocalcemia had higher incidences of in-hospital death and 6-month poor prognosis than those without [66 (25.00%) vs. 40 (4.04%), $P < 0.001$; 143 (54.17%) vs.127 (12.82%), $P < 0.001$]. In addition, the patients with hypocalcemia were older, and had higher APTTs, INRs, PTs, RBG, and frequency of severe or moderate injury, hypertension, diabetes, IVH, SAH, SDH, ICH, and neurosurgical intervention at admission compared with those without hypocalcemia (Table 1).

3.2. Lower serum calcium is associated with increased incidence of unfavorable 6-month prognosis

The patients with poor 6-month prognosis had a relatively low mean serum calcium level (Fig. 1A). The smooth plots revealed a nonlinear negative association between serum calcium and incidence of unfavorable 6-month prognosis (Fig. 1B). In particular, the incidence of an unfavorable 6-month prognosis decreased sharply [odds ratio (OR) 0.144; 95% confidence interval (95% CI) 0.09–0.23] with increasing serum calcium levels up to 2.18 mM/L (Table 2). When the serum calcium level exceeded the inflection point at 2.18 mmol/L, the incidence of unfavorable prognosis decreased slightly (OR: 0.65, 95% CI 0.43–0.98; Table 2).

3.3. Predictive models with serum calcium have superior performance for determining unfavorable 6-month prognosis

In the development cohort, univariate logistic regression analyses revealed that variables including the severity of injury mechanism, age, MAP, diabetes, smoking, contusion, IVH, SAH, SDH, PT, INR, APTT, platelet count, RBG, GCS level, and serum calcium level were linked to 6-month prognosis (Table S1). The variables retained in multivariate logistic regression and incorporated into the basic model for 6-month prognosis included age (OR 1.04; 95% CI 1.02–1.05), smoking (OR 1.71; 95% CI 1.05–2.79), GCS level (OR 3.28; 95% CI 1.81–5.93; moderate vs. < mild; OR 9.80; 95% CI 6.25–15.36; severe vs. mild), RBG (OR 1.09; 95% CI 1.03–1.15), INR (OR 2.78; 95% CI 0.70–10.95), IVH (OR 3.27; 95% CI 1.57–6.82; yes vs. no), SDH (OR 1.63; 95% CI 1.05–2.54; yes vs. no). Serum

Table 1
Baseline characteristics according to hypocalcemia level in the development cohort.

Variable		Hypocalcemia		
	Total (n = 1255)	No (n = 991)	Yes (n = 264)	*P* Value
Demographics and clinical variables				
Male gender, n (%)	971 (77.37%)	765 (77.19%)	206 (78.03%)	0.837
Age, meidan (IQR)	51.00 (34.00,64.00)	50.00 (33.00, 62.00)	54.00 (38.00, 67.00)	<0.001
Hypertension, n (%)	200 (15.94%)	162 (16.35%)	38 (14.39%)	0.499
Diabetes, n (%)	104 (8.05%)	84 (8.48%)	20 (7.58%)	0.729
Smoking, n (%)	321 (25.6%)	286 (28.9%)	35 (13.3%)	<0.001
Drink abuse, n (%)	150 (11.95%)	131 (13.22%)	19 (7.20%)	0.010
Neurosurgical intervention, n (%)	263 (20.96%)	188 (18.97%)	75 (28.41%)	0.001
Mean arterial pressure, median (IQR)	98.67 (90.00,110.00)	98.67 (90.00, 110.33)	98.00 (88.75, 109.17)	0.435
The severity of injury mechanism, n (%)				0.253
Mild	621 (49.48%)	502 (50.66%)	119 (45.08%)	
Moderate	193 (15.38%)	147 (14.83%)	46 (17.42%)	
Severe	441 (35.14%)	342 (34.51%)	99 (37.50%)	
Level on Glasgow coma scale scores, n (%)				<0.001
Mild (13–15 points)	846 (67.41%)	718 (72.45%)	128 (48.49%)	
Moderate (9–12 points)	132 (10.52%)	96 (9.69%)	36 (13.64%)	
Severe (3–8 points)	277 (22.07%)	177 (17.86%)	100 (37.88%)	
6-month unfavorable outcome after TBI, n (%)	270 (21.51%)	127 (12.82%)	143 (54.17%)	<0.001
In-hospital death	106 (8.45%)	40 (4.04%)	66 (25.00%)	<0.001
Imaging variables				
Intraventricular hemorrhage, n (%)	62 (4.94%)	42 (4.24%)	20 (7.58%)	0.039
Subarachnoid hemorrhage, n (%)	924 (73.63%)	711 (71.75%)	213 (80.68%)	0.003
Subdural hemorrhage, n (%)	773 (61.59%)	596 (60.14%)	177 (67.05%)	0.048
Extradural hemorrhage, n (%)	262 (20.88%)	214 (21.59%)	48 (18.18%)	0.260
Contusion, n (%)	1011 (80.56%)	781 (78.81%)	230 (87.12%)	<0.001
Laboratory results				
Prothrombin time, meidan (IQR)	11.30 (10.70,12.00)	11.20 (10.60, 11.80)	11.80 (11.10, 12.50)	<0.001
INR, median (IQR)	0.98 (0.93,1.04)	0.97 (0.93, 1.03)	1.03 (0.97, 1.09)	<0.001
ATPP, median (IQR)	24.40 (21.90,27.10)	24.20 (21.70, 26.40)	25.80 (23.10, 29.40)	<0.001
Fibrinogen, meidan (IQR)	2.22 (1.79,2.73)	2.25 (1.81, 2.73)	2.12 (1.61, 2.69)	0.023
Platelet count, meidan (IQR)	214.00 (178.00,255.00)	219.00 (185.00, 257.00)	189.00 (154.00, 234.00)	<0.001
Random blood glucose, meidan (IQR)	8.00 (6.69,9.84)	7.90 (6.61, 9.61)	8.57 (7.04, 11.08)	<0.001

ATPP, activated partial thromboplastin time; INR, international normalized ratio; IQR, interquartile range.

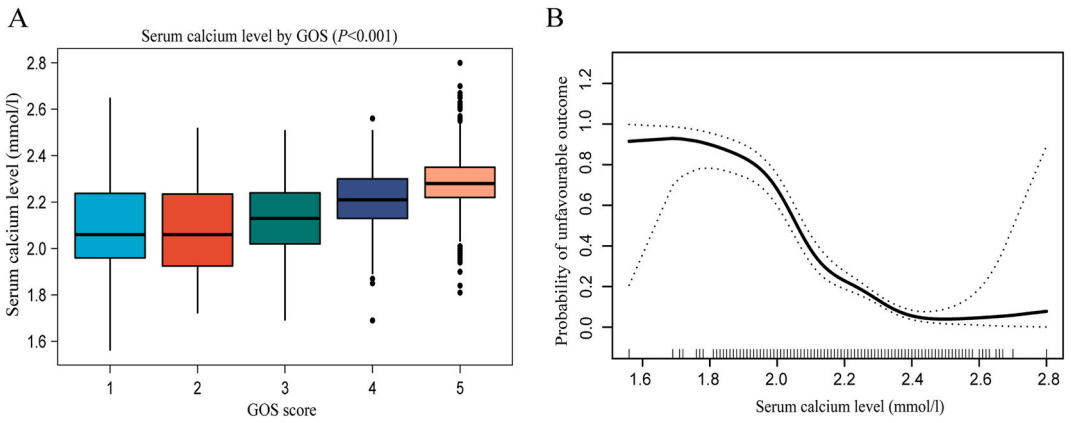

Fig. 1. Serum calcium at admission is negatively associated with an unfavorable 6-month prognosis.
(**A**) Serum calcium level in each Glasgow outcome scale (GOS) group. (**B**) Relationship between serum calcium levels and an unfavorable 6-month prognosis. Results were adjusted by age, male, level on Glasgow Coma Scale scores, and RBG, INR, IVH, and SDH.

Table 2
The associations of serum calcium level with 6-month unfavorable outcomes.

	6-month unfavorable outcomes			
	Unadjusted OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
Serum calcium (per increasing 1SD)	0.36 (0.31,0.42)	<0.001	0.41 (0.34,0.51)	<0.001
Serum calcium <2.18 (per increasing 1SD)	0.14 (0.09,0.23)	<0.001	0.23 (0.13,0.41)	<0.001
Serum calcium ≥2.18 (per increasing 1SD)	0.62 (0.43,0.90)	0.012	0.65 (0.43,0.98)	0.041

Adjustment by age, male, smoking, level on Glasgow Coma Scale score, random blood glucose, international normalized ratio, subdural hemorrhage and intraventricular hemorrhage.

calcium [Ca/CaSD (OR 0.41; 95% CI 0.34–0.51)] at admission and the variables in the basic model were incorporated into calcium for prognosis (Table S2).

The performance of four predictive models (i.e., single indicators: GCS level and serum calcium; combined models: basic and calcium model) in predicting the 6-month prognosis was compared using ROC curves. Two single indicators, serum calcium (AUC: 0.746, 95% CI 0.709–0.783) and GCS level (AUC: 0.768, 95% CI 0.737–0.799) showed no significant difference from each other and both of them exhibited acceptable prognostic performance (Fig. 2A). The calcium model exhibited the best discrimination for 6-month

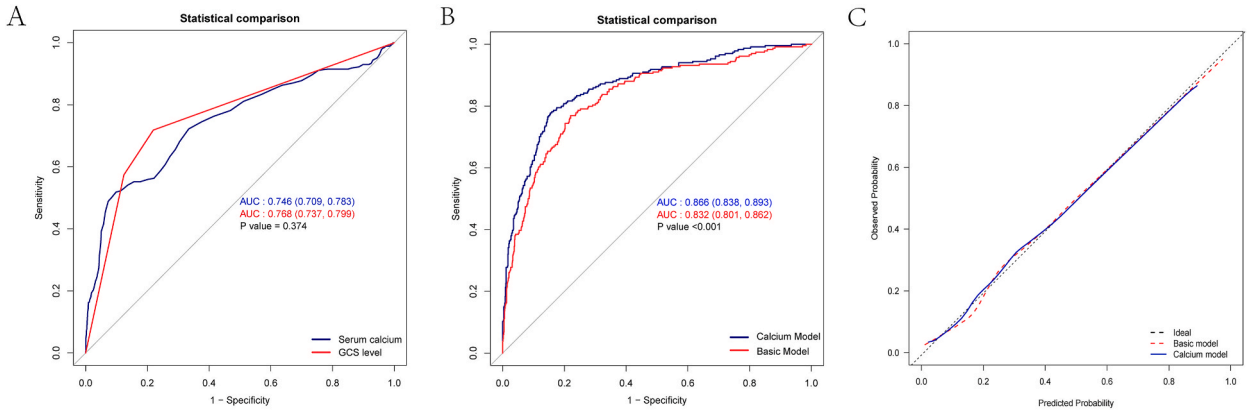

Fig. 2. Discrimination and calibration of the models for a 6-month prognosis in the development cohort.
Receiver operating characteristic (ROC) curve analysis was performed for GCS level, serum calcium, the basic model and calcium model, to predict an unfavorable 6-month prognosis. **(A)** Statistical comparison between serum calcium (blue line) and GCS level (red line). **(B)** Statistical comparison between the calcium model (blue line) and basic model (red line). **(C)** Calibration curves for the 6-month prognosis. The ideal is the theoretical curve, and the red and blue lines are the bias-corrected curves of the basic model and calcium model, respectively. The abscissa and ordinate are the predicted and actual probability values. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

prognosis (AUC: 0.866, 95% CI 0.801–0.893) compared with the individual GCS and serum calcium models, and the basic model (AUC: 0.832, 95% CI 0.801–0.862). The calcium model for 6-month prognosis exhibited better sensitivity (77.78% vs. 76.92%) and higher specificity (84. 45% vs. 77.99%) compared with the basic model (Fig. 2A and B). Calibration curves were used to evaluate the performance of these models further and showed that both the calcium model and the basic model exhibited good calibration (Fig. 2C).

3.4. External validation

In the external validation cohort, 719 out of 1377 (52.21%) patients with primary TBI were included (Fig. S1). Consistently, the patients with hypocalcemia had a higher incidence of 6-month poor prognosis than those without (Table S3). For clinical application, a calcium nomogram derived from the calcium model was constructed (Fig. 3). In the nomogram, every predictor was assigned a score, and the total score was obtained by linearly combining the points of each predictor in the range of 10–90% to assess the risk of an unfavorable 6-month prognosis.

Consistent with the development cohort, serum calcium (AUC: 0.783, 95% CI 0.744–0.821) and GCS levels (AUC: 0.776, 95%CI 0.743–0.809) also showed acceptable predictive performance in the validation cohort (Fig. 4A). The calcium nomogram derived from the calcium model (AUC: 0.875, 95% CI 0.847–0.904) maintained optimal discrimination for the 6-month prognosis compared with the models involving GCS level and serum calcium, and the basic model (AUC: 0.842, 95% CI 0.801–0.862) (Fig. 4A and B). The calcium nomogram and basic model for the 6-month prognosis maintained excellent calibration (Fig. 4C).

Finally, subgroup analysis revealed that the association between lower serum calcium level at admission and the 6-month prognosis was robust in a wide spectrum of patients with TBI (Fig. 5). A significant interaction was observed between serum calcium and GCS level in 6-month unfavorable outcomes (Fig. 5).

4. Discussion

This multicenter retrospective cohort study explored the association between serum calcium levels and unfavorable prognosis after primary TBI. The patients with a 6-month unfavorable prognosis had significantly lower serum calcium levels than those without. Lower serum calcium level at admission is an independent risk factor for a 6-month unfavorable prognosis in a wide spectrum of patients with TBI. When the serum calcium at admission is below 2.18 mM/L, the incidence of a 6-month unfavorable prognosis decreases sharply with the increasing serum calcium levels up to 2.18 mM/L. We introduced and externally validated a calcium nomogram for 6-month prognosis following TBI. The calcium nomogram maintained excellent performance in discrimination and

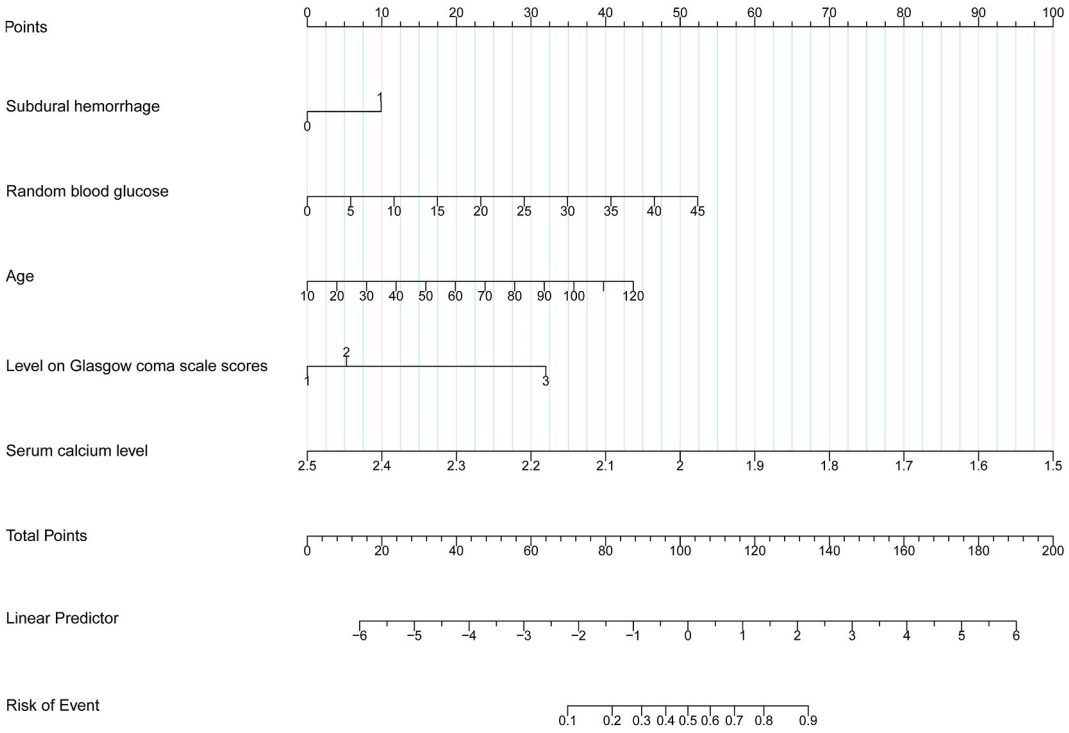

Fig. 3. A calcium model nomogram predicts an unfavorable 6-month prognosis.
Points for each parameter are assigned by corresponding values from the point axis to calculate a patient's probability for unfavorable 6-month prognosis. The sum of the points is plotted on the total point axis. The patient's 6-month prognosis probability is the value at a vertical line from the corresponding total points.

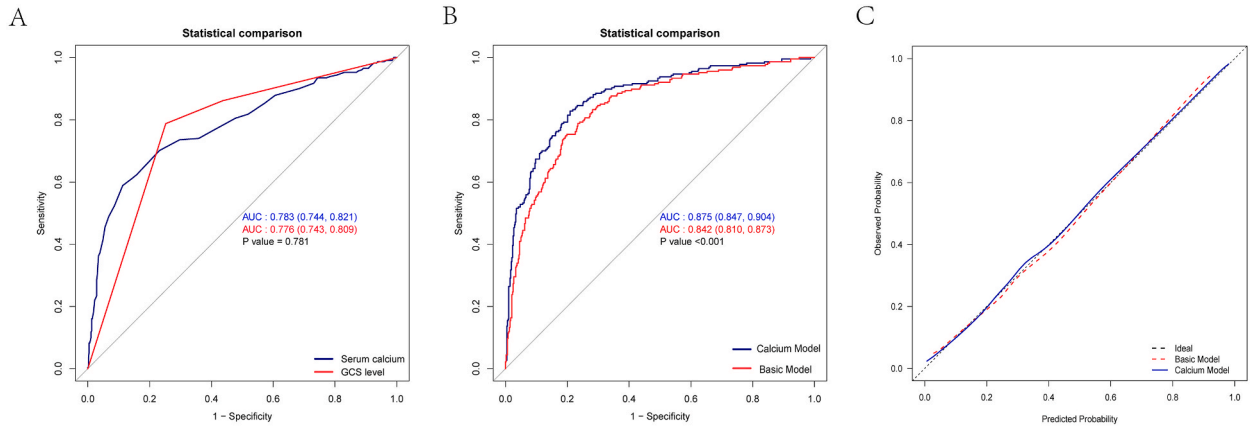

Fig. 4. Discrimination and calibration of the nomograms for the 6-month prognosis in the validation cohort.
Receiver operating characteristic (ROC) curve analysis was performed for GCS level, serum calcium, the basic model and calcium nomogram, to predict the 6-month prognosis. **(A)** Statistical comparison between serum calcium (blue line) and GCS level (red line). **(B)** Statistical comparison between the calcium nomogram (blue line) and basic model (red line). **(C)** Calibration curves for the 6-month prognosis. The ideal is the theoretical curve, and the red and blue lines are the bias-corrected curves of the basic model and calcium nomogram, respectively. The abscissa and ordinate are the model's predicted and actual probability values. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

calibration in the externally validated cohort.

Serum calcium levels can predict prognoses in various diseases, including COVID-19, nasopharyngeal carcinoma, acute pulmonary embolism, and stroke [7,12,23–25]. A lower serum calcium level can lead to bleeding, exacerbating secondary injury [7], and at admission is an independent risk factor for high hematoma volume and acute progression after spontaneous or traumatic ICH [12,26]. Karawan et al. noted that mild admission hypocalcemia is associated with better neurological status at discharge in patients with isolated severe TBI [27]. Our study also reports on the prognostic predictive value of serum calcium in patients with TBI. However, it is worth noting that Karawan et al. also point out their own limitations (single-center retrospective study and relatively small sample size) and cannot exclude the effect of unmeasured or incompletely measured confounders. In addition, Karawan et al. focused on neurological outcome at discharge (favorable neurological status defined by a modified Rankin Scale score of 0–2) [27], whereas we focused on prognosis at 6 months. Although our study differs from that of Karawan et al. in many ways, such as design of the study, definition of outcomes, and time points for recording prognosis, our results both found a predictive value of calcium ions on the prognosis of patients with TBI.

Regarding the mechanism, calcium ion is a critical cofactor in the coagulation cascade. Lower serum calcium may result in coagulopathy. Fukuda et al. reported that low serum calcium level is remarkably associated with prolonged blood clotting time and tendency to bleed, leading to traumatic hematoma progression [28]. Hypocalcemia also increases vascular tone and blood pressure, contributing to the acute progression of traumatic intraparenchymal hemorrhage [29,30]. As an important malignant progression event, hematoma likely results in acute exacerbation and long-term prognosis after primary TBI [31,32]. Our latest in vitro TBI model showed that calcium inflow in cortical neurons is attenuated after mechanical injury, and enhancing calcium inflow through L-type calcium channels may rescue neuronal damage [33]. We speculated that lower serum calcium levels might reflect a low calcium level in the extracellular environment, which would cause decreased calcium ion inflow in neurons and exacerbate neuronal death or persistent neuronal degeneration at the trauma site. These reasons may explain the predictive value of lower serum calcium for long-term unfavorable prognosis after primary TBI.

In addition to serum calcium, several variables, including age, GCS level, SDH, and RBG, were associated with the 6-month prognosis. The effect of advanced age on long-term poor recovery in patients with TBI has been widely reported [34]. The GCS has been validated as an excellent indicator for monitoring and predicting the prognosis of patients with TBI [35]. The interaction test indicated that lower serum calcium was more significantly associated with a 6-month unfavorable prognosis in mild patients than in moderate to severe patients. A recent study from Karawan et al. also demonstrated the possible higher prognostic value of calcium in patients with a Glasgow Coma Scale >8 at admission, which partially coincides with our finding [27]. Therefore, at admission, attention and follow-up visits should be given to mild patients with lower serum calcium levels. INR as a coagulation factor has been shown to correlate with the prognostic outcome of TBI [36], and this was confirmed in our study. Persistent high blood glucose levels after brain trauma can cause complications, including reduced resistance, infections, and hyperosmolarity [37]. High RBG is a significant serological risk factor for poor prognosis in pediatric patients with TBI [38]. Our present results indicate that high RBG is associated with long-term unfavorable prognosis after TBI.

Given the heterogeneity of TBI, prognosis is difficult to accurately predict using a single predictor [39]. In recent years, some multivariate combined models for the unfavorable prognosis of TBI have been reported. Rubin et al. reported a similar combined model (AUC = 0.86) with hospital admission characteristics, injury severity characteristics, and physiological monitoring for the 6-month prognosis of severe TBI patients [40]. Regarding predictors, the same variables, such as age, GCS level, and RBG are also

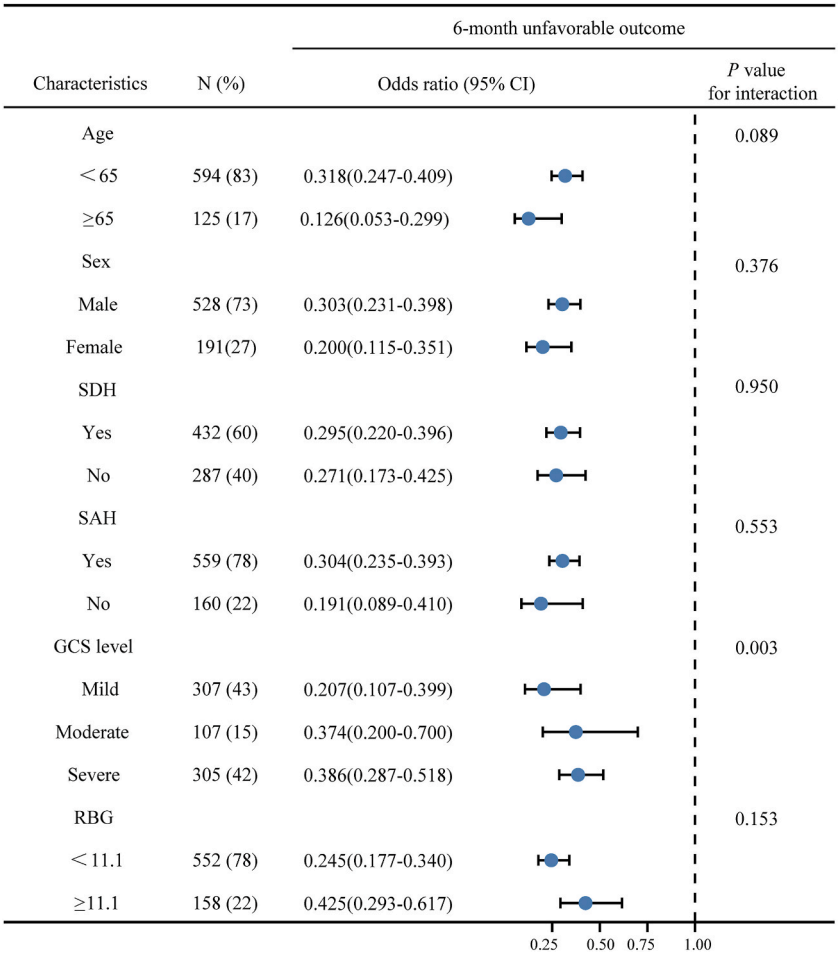

Fig. 5. Odds ratio (OR) for an unfavorable 6-month prognosis in subgroups of patients with TBI, and interaction test of the stratification variables and serum calcium at admission.

included in our combined models/or nomograms. Given the simplicity of the nomogram, other predictors, including smoking, INR, and an imaging variable of IVH and SDH were eliminated from the final 6-month calcium nomogram because they did not significantly improve the discrimination. Including serum calcium levels at admission in the final combined models substantially improved their performance for the 6-month prognosis (AUC = 0.875). The calcium nomograms derived from the calcium models maintained excellent discrimination and calibration for the external validation cohort. Compared with previous predictive models, the calcium model/nomogram provides more accurate prediction for the 6-month prognoses using fewer and more convenient routine indicators at admission. Given their convenience and robustness, the calcium nomogram can provide neurosurgeons with additional recommendations regarding patient prognosis, such as reduced unnecessary management and treatment for patients with a low probability of poor prognosis.

A model reported by Rubin et al. has been developed only for patients with severe TBI. This model may not be suitable for patients suffering from mild or moderate TBI because of its bias. In the present work, a calcium nomogram was derived from a large multicenter retrospective cohort and exhibited a high and stable performance for predicting the 6-month prognosis. In clinical practice, the calcium nomogram can be used by neurosurgeons to make rapid and accurate initial predictions of long-term prognosis, thus providing support for the individualized management and treatment of patients with TBI. Patients with a high risk of an unfavorable 6-month prognosis could benefit from early intervention, intensive surveillance, and follow-up. Meanwhile, patients with a low risk of a 6-month unfavorable prognosis may avoid excessive medical treatment and unnecessary intensive monitoring.

The strengths of our study include a large sample size, multicenter design, externally validated design, well-characterized patients with TBI, and quantitative measurements of pathological and clinical variables. Given the completeness of data, the cohorts allow for robust statistical modeling. Our study focused on the relationship between admission serum calcium and long-term unfavorable prognosis after primary TBI to provide an objective interpretation of prognoses rather than subjective clinical scores, such as the GCS. The final calcium nomograms with several crucial and easily accessible clinical indicators at admission provide an accurate and robust prediction for the 6-month prognosis. In the early stages of TBI, the calcium nomogram can serve as a practical and handy tool for neurosurgeons in their preliminary judgment of an unfavorable prognosis, and provide support for the individualized management and

treatment of patients with TBI.

Several limitations must be considered in the interpretation of these findings. First, considering the retrospective design, this study is subject to selection and information biases inherent in its design. Although we used a multicenter study design and externally validated predictive models to minimize the effect of selection bias, a prospective multicenter cohort study is warranted to validate the value of the calcium nomograms for 6-month prognosis. Second, ionized calcium reflects biological functions better than serum calcium. However, the present reports lack data on ionized calcium. Given the good correlation between serum calcium levels and ionized calcium levels [25,26], low serum calcium levels may similarly reflect abnormal biological function. Third, it is notable that the dataset of this study did not include those patients who did not have intracranial hemorrhage at baseline CT. Therefore, to be precise, we are evaluating the predicted prognosis of serum calcium for more severe patients. Whether serum calcium levels can help diagnose injury was not explored in depth in this study, and for the present results, serum calcium may be a potentially valuable diagnostic indicator of injury that needs to be validated by further studies. Finally, calcium nomograms should be used with caution in clinical decision-making because they are not a substitute for a surgeon's clinical assessment of a patient.

5. Conclusion

Lower serum calcium level at admission is associated with 6-month prognosis following TBI. Integrating serum calcium in multivariate predictive models optimizes their performance for the 6-month prognosis. Using several hospital admission characteristics, the calcium nomogram provides a practical and handy tool for unfavorable long-term prognosis to support the individualized treatment and management of patients in the early stages of TBI.

Author statement

Tian Li, MD: Data collection and statistical analysis, drafted the manuscript for intellectual content, Weiqiang Chen, MD, PhD: Design and conceptualized study, revision of manuscript, Dongzhou Zhuang, MD: Data collection and statistical analysis, revision of manuscript, Shirong Cai, MD: Data collection and imaging analysis, revision of manuscript, Faxiu Ding, MD: Data collection, imaging analysis, and revision of manuscript, Fei Tian, MD: Data collection, revision of manuscript, Mindong Huang, MD: Data collection, revision of manuscript, Lianjie Li, MD: Data collection, revision of manuscript, Kangsheng Li, MD, PhD: Design and conceptualized study, revision the manuscript for intellectual content manuscript for intellectual content, Jiangtao Sheng, MD: Design and conceptualized study, Data statistical analysis, drafted the manuscript for intellectual content manuscript for intellectual content.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Ethics approval

This study was approved by the ethics committees of the four hospitals mentioned above. The ethical approval number (No. 2020-042) was available for all ethics committees. All procedures in this study followed the ethical standards and regulations in the appropriate version of the Declaration of Helsinki.

Consent to participate

Given the anonymity of the data from the retrospective cohorts, the requirement for informed consent was waived.

Consent for publication

Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18475.

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