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# Direct bilirubin: A predictor of hematoma expansion after intracerebral hemorrhage

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ABSTRACT

*Background:* Previous evidence demonstrated that several biomarkers involved in the pathological process of coagulation/hemostasis dysfunction, impairment of brain vascular integrity and inflammation are associated with hematoma expansion (HE) after intracerebral hemorrhage (ICH). We aimed to explore whether there were unreported laboratory biomarkers associated with HE that were readily and commonly available in clinical practice.
*Methods:* We retrospectively analyzed consecutive acute ICH patients from 2012 to 2020 with admission laboratory tests and baseline and follow-up computed tomography (CT) scans. Univariate and multivariate regression analyses were used to evaluate associations between conventional laboratory indicators and HE. The results were verified in a prospective validation cohort. The relationship of candidate biomarker and 3-month outcomes was also investigated and mediation analysis was undertaken to determine causal associations among candidate biomarker, HE and outcome.
*Results:* Of 734 ICH patients, 163 (22.2%) presented HE. Among the included laboratory indicators, higher direct bilirubin (DBil) was associated with HE (adjusted odds ratio [OR] of per 1.0 μmol/L change 1.082; 95% confidence interval [CI] 1.011–1.158). DBil >5.65 μmol/L was a predictor of HE in validation cohort. Higher DBil was also associated with poor 3-month outcomes. The mediation analysis indicated that the association of higher DBil and poor outcomes was partially mediated by HE.
*Conclusions:* DBil is a predictor of HE and poor 3-month outcomes after ICH. DBil's metabolic process and involvement in the pathological mechanism of HE are likely to contribute to the association between DBil and HE. Interventions targeting DBil to improve post-ICH prognosis may be meaningful and worthy of further exploration.


## 1. Introduction

Intracerebral hemorrhage (ICH) accounts for 10%–20% of all strokes [1,2] and is associated with high risks of mortality and disability [3]. Hematoma expansion (HE) occurs in approximately 1/3 of patients after ICH and is a strong predictor of poor post-ICH outcomes [4,5]. Predicting HE in a timely manner contributes to the early identification of HE and the implementation of targeted interventions, with the possibility of improving prognosis. Therefore, there is a crucial need to identify feasible HE-related biomarkers to achieve better management of ICH.

Quite a few studies have been dedicated to exploring potential predictors of HE to develop possible intervention targets. Among them, the CT angiography (CTA) spot sign is regarded as a robust predictor of HE [6], although its availability may be limited to some extent, and individual judgment may lead to subjective bias. Biomarkers evaluated by laboratory tests have the advantages of simplicity, convenience and accessibility and may provide beneficial clues to unravel the underlying mechanisms. Some proteins, such as hemoglobin, matrix metalloproteinase-9 (MMP-9) and C-reactive protein (CRP), involved in the pathways of coagulation/hemostasis dysfunction, impairment of brain vascular integrity and inflammation were reported to be relevant to HE [7-13]. In addition, some electrolytes, namely, magnesium and calcium, have been associated with impaired coagulation status, which could affect HE [14,15]. Whether there are other biomarkers readily and commonly available from laboratory tests that are able to predict HE remains attractive to further study.

The purpose of our study was to investigate the optimal unreported laboratory biomarkers associated with HE that are quick and easy to

*Abbreviations:* DBil, direct bilirubin; GCS, Glasgow Coma Scale; ICH, intracerebral hemorrhage; IVH, intraventricular hemorrhage; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale.

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obtain to identify HE in a timely manner and provide evidence for developing new interventions for improving prognosis.

## 2. Methods

### 2.1. Patients

We retrospectively extracted relevant data from our prospective cohort of consecutive patients with ICH treated within 24 h after symptom onset from January 2012 to November 2020 in the neurology department of the hospital. The diagnosis of ICH was based on the World Health Organization definition of stroke [16] combined with imaging findings of ICH on the computed tomography (CT) scan. Patients who were younger than 18 years, had primary IVH, had missing laboratory tests on admission, and had ICH due to trauma, tumors, or hemorrhagic transformation of cerebral infarction with or without thrombolytic therapy were excluded. To evaluate hematoma expansion, we also excluded patients without baseline CT scans within 6 h of symptom onset and follow-up CT scans within 48 h of baseline.

We also prospectively enrolled consecutive patients with ICH treated from December 2020 to November 2021 in the same department with baseline CT and laboratory tests within 6 h after symptom onset as the validation cohort. All patients underwent a follow-up CT within 24–48 h from baseline or earlier if the patient clinically deteriorated or was ready for surgery. Patients who were younger than 18 years, had primary IVH, had mechanical ventilation and were unable to undergo follow-up CT, and had ICH due to trauma, tumors, or hemorrhagic transformation of cerebral infarction with or without thrombolytic therapy were excluded.

The study was approved by the institutional ethics board of Huazhong University of Science and Technology, and informed consent was obtained from all participants (Clinical Trial Registration-URL: http://www.chictr.org.cn. Unique identifier: ChiCTR-ROC-2000039365).

### 2.2. Data collection

The following baseline data were obtained for the enrolled patients: (1) demographic data, including age and sex; (2) past medical and medication history, including hypertension, diabetes mellitus, coronary heart disease, atrial fibrillation, anticoagulant agents, and previous stroke, as well as chronic liver disease, antiplatelet agents, and statin use in the validation cohort; (3) imaging data, including the location and volume of the hematoma (measured by the ABC/2 method [17]), the presence of IVH or subarachnoid space extension, cerebral angiography [CTA, magnetic resonance angiography (MRA), or digital subtraction angiography (DSA)]; and (4) laboratory tests, including biochemistry, routine blood, coagulation function, and infection biomarkers (CRP) (considering clinical practicality, we used well-known conventional laboratory indicators); (5) other parameters, including blood pressure on admission, the pre-ICH modified Rankin Scale (mRS), the National Institutes of Health Stroke Scale (NIHSS) score and the Glasgow Coma scale (GCS) score on admission; and (6) therapy, including conservative treatment, ventricular drainage or minimally invasive hematoma evacuation. Laboratory values were obtained immediately on admission and conducted in the department of laboratory medicine in our hospital. All imaging was conducted in our hospital except for the baseline CT scans of 13 patients in the validation cohort (conducted in other institutions), and all the images were reviewed by two neurologists referring to reports from an expert radiologist. CT image acquisition was performed on a GE Discovery CT750 HD by scanning from the base of the skull to the vertex using an axial technique. The CT protocols were as follows: 120 kV, automatic tube current modulation (300 mAs), 5 mm section interval, and 5 mm section thickness. HE was defined as absolute growth >12.5 mL or relative growth >33% from baseline to follow-up CT.

The follow-up was conducted by a telephone interview. The evaluation of patients' prognoses was blinded to their clinical data. The clinical outcome was measured by mRS at the 3-month follow-up, and an unfavorable outcome was defined as mRS >3.

### 2.3. Statistical analysis

Data are reported as the mean ± SD, median (IQR) for continuous variables, or n (%) for categorical variables. Pearson, $\chi^2$ and Fisher exact-tests were used to compare group data for categorical variables. Student's t-test or the Mann-Whitney *U* test was applied to analyze continuous variables between two groups. Several continuous variables had missing data [101 (13.8%) for CRP; 10 (1.4%) for glomerular filtration rate (GFR); 25 (3.4%) for glucose; 65 (8.9%) for magnesium; 49 (6.7%) for prothrombin time (PT), fibrinogen, activated partial thromboplastin time (APTT), thrombin time (TT); 21 (2.9%) for international normalized ratio (INR); 48 (6.5%) for triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL)], but the rates of missing data were <15% and were imputed as the mean value of the remaining available data [18]. The variables with *P* values<0.1 from the univariate analysis and variables previously shown to be predictive of HE were included in the multivariate analysis using Wald forward logistic regression model to evaluate the association between the laboratory indicators and HE and then to screen the candidate biomarkers of HE. Then the restricted cubic spline (RCS) and receiver operating characteristic (ROC) analyses were performed for the candidate biomarkers which were significant in both the univariate and multivariate analysis. The RCS model with three knots was used to test the linear association between candidate biomarker levels and HE. ROC analysis was performed to determine the cutoff values of candidate biomarkers for predicting hematoma expansion. The diagnostic value of candidate biomarkers for HE was assessed by calculating the area under the curve (AUC) of the ROC curve in the validation cohort. An additional logistic regression model assessed the association of candidate biomarker levels with poor 3-month clinical outcomes after adjusting for variables with *P* values<0.1 from the univariate analysis. A mediation analysis was performed to estimate whether HE (as the mediator) was the driving factor for any relationship between candidate biomarkers (independent variable) and poor outcomes (dependent variable) by regression analysis of all three variables together [19]. Two-sided *P* values <0.05 were considered statistically significant. All analyses were performed with IBM SPSS software, version 26 (SPSS Inc., Chicago, IL, USA) and R software version 4.1.1 (The R Foundation for Statistical Computing, Vienna, Austria. http://www.r-project.org). We prepared this article using STROBE, which is the guideline for reports of cohort studies.

## 3. Results

A total of 1705 patients with primary ICH were screened, and 734 patients were included according to the inclusion and exclusion criteria (male: 66.2%; mean age: 56.3 ± 12.2 years, Fig. 1). There were 163 (22.2%) patients presenting HE. In addition to infratentorial ICH and white blood cells, the other baseline characteristics were similar between the study participants and the excluded patients (Supplementary Table S1).

Intergroup differences between patients with HE and those without are shown in Table 1. Patients with HE had shorter time intervals from symptom onset to the first CT scan, more frequent presence of IVH, lower platelet counts, higher glucose levels and worse NIHSS and GCS scores ($P < 0.05$). In the multivariate analysis, as shown in Table 2, time from symptom onset to first CT scan, presence of IVH, NIHSS on admission and direct DBil were associated with HE. Therefore, DBil was regarded as a candidate biomarker of HE. The dose–response relationship between the DBil level and the risk of HE was further demonstrated with RCS (P for nonlinearity = 0.465, Fig. 2A), and DBil predicted the probability of HE in a linear, dose-dependent relationship ($r = 0.243$,

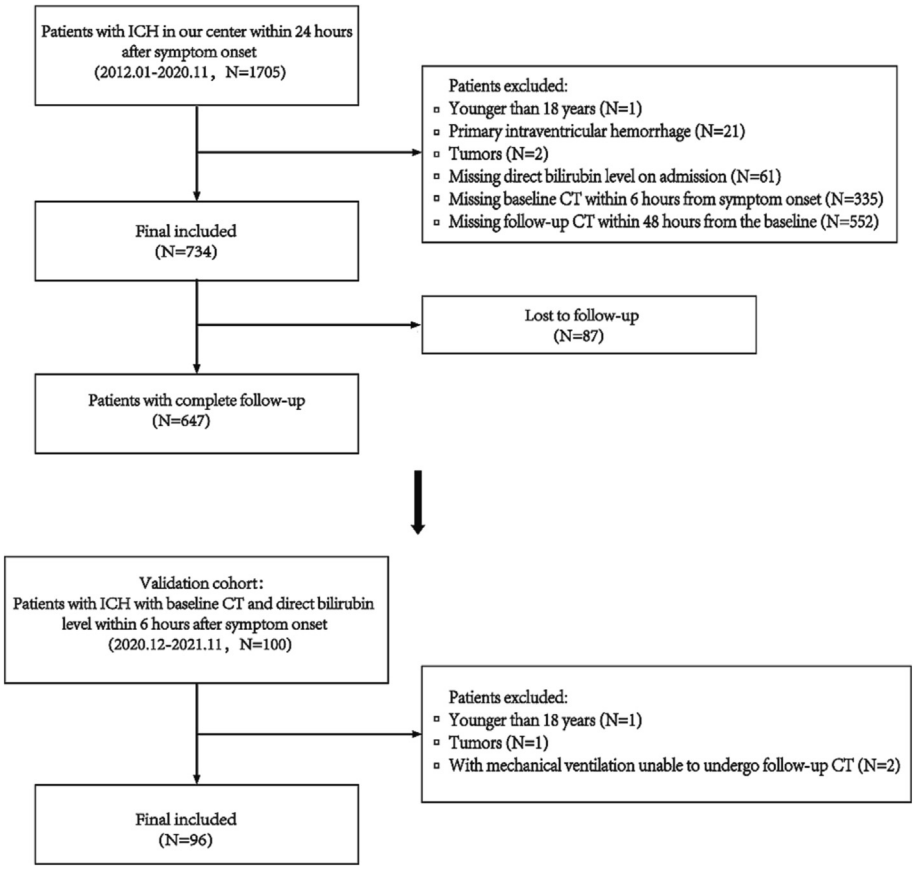


**Fig. 1.** Flowchart of study patients. ICH, intracerebral hemorrhage.

$P < 0.001$, Fig. 2B). According to ROC analysis, we used 5.65 μmol/L as the cutoff value.

A total of 96 patients were included in the validation cohort (male: 74.0%; mean age: 55.2 ± 12.9 years, Fig. 1). There were 35 (36.5%) patients presenting HE. Intergroup comparisons between patients with HE and those without are shown in Supplementary Table S2. The multivariate logistic regression model revealed that DBil >5.65 μmol/L was an independent predictor of HE (adjusted OR 4.476; 95% CI, 1.404–14.270; $P = 0.011$ in model 1 and 3.871; 95% CI, 1.283–11.675; $P = 0.016$ in model 2, Table 3). In the ROC curve, the AUC was 0.656, with a 95% CI of 0.535–0.776 ($P = 0.013$, Fig. 3).

A total of 87 patients were lost to 3-month follow-up due to providing the wrong phone number or failure to answer the phone call. We compared the baseline characteristics between the group with complete follow-up and the group lost to follow-up. Lobar ICH, deep ICH and the presence of IVH were significantly different between the two groups, while the other characteristics showed no differences (Supplementary Table S3). Intergroup differences between patients with favorable and unfavorable outcomes are shown in Supplementary Table S4. In the evaluation of the relationships between DBil levels and 3-month outcomes, we identified an association of higher DBil levels with increased odds of poor 3-month outcomes (adjusted OR per 1.0 μmol/L change 1.145; 95% CI 1.058–1.245; $P = 0.001$, Supplementary Table S4). When regressing DBil, HE, and outcome together, the mediation analysis revealed that HE significantly mediated 21.69% of the association between admission DBil level and poor outcome (Fig. 4).

## 4. Discussion

In this study, we investigated the association between biomarkers from conventional laboratory tests and HE after ICH and found that DBil was an independent predictor of HE with a dose–response relationship. In addition, we verified that the DBil level was associated with a 3-month unfavorable outcome after ICH, and HE appeared to partially mediate this relationship.

It is of great clinical significance to predict and control HE after ICH considering the disastrous effect of HE on the post-ICH prognosis. Exploring optimal biomarkers is clearly a priority. Previously reported biomarkers indicated that dysregulation of pathways, including coagulation/hemostasis, cerebrovascular integrity and inflammation, is critical for HE. Regarding proteins, a low level of fibrinogen and low hemoglobin may induce HE due to an impairment of coagulation and hemostasis functions [9,11]. Plasma cellular fibronectin (c-Fn) and MMP-9 were demonstrated to have favorable predictive value for HE resulting in vascular injury [10,13]. Interleukin-6 (IL-6), CRP and lactate dehydrogenase (LDH) related to the inflammatory response were revealed by HE [7,8,10]. Regarding electrolytes, low serum levels of magnesium [15] and calcium [14] have been reported to be associated with HE, with evidence of coagulopathy. Glucose that are involved in carbohydrate and amino acid related metabolic pathways has been found to be associated with HE [20] and bradykinin-mediated hemostasis inhibition was suggested to contribute to the relationship [21]. In our study, although not significant in multivariate analysis, patients with HE showed lower magnesium and higher glucose levels than did non-HE patients in univariate analysis ($P < 0.05$). When we regard hematoma growth as a continuous variable similar to Liotta EM et al. and Liotta EM et al. and use linear regression to investigate the association between glucose as well as magnesium and hematoma growth, we found higher glucose was significant associated with greater hematoma growth in the univariate and multivariate analysis, while lower magnesium was significant in the univariate analysis and

**Table 1**
Comparison between ICH patients with and without hematoma expansion.

| | Total ($n$ = 734) | Non-HE ($n$ = 571) | HE ($n$ = 163) | P Value |
|---|---|---|---|---|
| Age, y | 56.3 ± 12.2 | 56.0 ± 12.1 | 57.2 ± 12.5 | 0.281 |
| Male sex | 486 (66.2%) | 372 (65.1%) | 114 (69.9%) | 0.254 |
| Hypertension | 526 (71.7%) | 414 (72.5%) | 112 (68.7%) | 0.343 |
| Diabetes mellitus | 73 (9.9%) | 54 (9.5%) | 19 (11.7%) | 0.408 |
| Coronary heart disease | 43 (5.9%) | 32 (5.6%) | 11 (6.7%) | 0.583 |
| Atrial fibrillation | 6 (0.8%) | 4 (0.7%) | 2 (1.2%) | 0.619 |
| Oral anticoagulation | 19 (2.6%) | 13 (2.3%) | 6 (3.7%) | 0.399 |
| Previous stroke | 112 (15.3%) | 92 (16.1%) | 20 (12.3%) | 0.229 |
| Smoking habit | 270 (36.8%) | 206 (36.1%) | 64 (39.3%) | 0.457 |
| Drinking habit | 242 (33.0%) | 191 (33.5%) | 51 (31.3%) | 0.605 |
| Initial ICH volume, mL | 18.1 (8–36.1) | 17.6 (8.7–33.6) | 18.5 (7.9–36.8) | 0.734 |
| Lobar ICH | 200 (27.2%) | 157 (27.5%) | 43 (26.4%) | 0.778 |
| Deep ICH | 551 (75.1%) | 424 (74.3%) | 127 (77.9%) | 0.341 |
| Infratentorial ICH | 41 (5.6%) | 29 (5.1%) | 12 (7.4%) | 0.263 |
| Subarachnoid space extension | 70 (9.5%) | 49 (8.6%) | 21 (12.9%) | 0.099 |
| Presence of IVH | 244 (33.2%) | 172 (30.1%) | 72 (44.2%) | <0.001 |
| Midline shift | 144 (19.6%) | 105 (18.4%) | 39 (23.9%) | 0.116 |
| Time from symptom onset to first CT scan, h | 4 (2–6) | 4 (2–6) | 3 (2–5) | <0.001 |
| Admission SBP, mmHg | 156.84 ± 24.65 | 156.39 ± 24.66 | 158.39 ± 24.61 | 0.363 |
| Admission DBP, mmHg | 91.39 ± 16.90 | 91.22 ± 17.03 | 91.97 ± 16.48 | 0.619 |
| WBC, 10^9/L | 10 (7.6–12.8) | 9.9 (7.6–12.5) | 10.4 (7.6–13.2) | 0.448 |
| Neutrophil, 10^9/L | 8.2 (5.7–10.9) | 8 (5.7–10.8) | 8.7 (5.7–11.6) | 0.235 |
| Neutrophil percentage, % | 82.3 (74.0–88.3) | 82.3 (73.6–88.0) | 82.7 (75.0–89.3) | 0.074 |
| RBC, 10^12/L | 4.7 (4.3–5.0) | 4.7 (4.3–5.0) | 4.6 (4.3–5.1) | 0.844 |
| Hemoglobin, g/L | 141 (129–152) | 141 (129–151) | 141 (130.5–152) | 0.464 |
| Platelet count, 10^9/L | 199.8 ± 60.9 | 203 ± 60.6 | 188.5 ± 61.1 | 0.007 |
| ALT, U/L | 16 (12−23) | 16 (12–23) | 17 (13–25) | 0.301 |
| AST, U/L | 20 (16–25) | 20 (16–25) | 21 (17–25) | 0.126 |
| Total Cholesterol, mmol/L | 4.4 (3.8–5) | 4.4 (3.8–5.1) | 4.4 (3.8–4.9) | 0.274 |
| Triglyceride, mmol/L | 1.1 (0.8–1.5) | 1.1 (0.8–1.5) | 1.1 (0.8–1.4) | 0.851 |
| HDL, mmol/L | 1.2 (1–1.4) | 1.2 (1–1.4) | 1.2 (1–1.4) | 0.572 |
| LDL, mmol/L | 2.8 (2.2–3.2) | 2.8 (2.2–3.3) | 2.7 (2.2–3.2) | 0.721 |
| Total bilirubin, μmol/L | 12.9 (8.8–17.0) | 12.8 (8.8–16.7) | 13.2 (8.9–17.8) | 0.528 |
| Direct bilirubin, μmol/L | 3.7 (2.5–5.1) | 3.6 (2.5–4.9) | 3.8 (2.7–5.8) | 0.087 |
| Albumin, g/L | 42.7 (39.9–45.3) | 42.7 (39.8–45.4) | 42.7 (40.2–45) | 0.972 |
| LDH, U/L | 211 (180.0–234.0) | 212 (180.0–233.5) | 208 (176.0–236.5) | 0.595 |
| Creatinine, μmol/L | 72.5 (59.0–89.0) | 72 (58.5–89.0) | 75 (59.0–91.0) | 0.280 |
| GFR, ml/min/1.73 m^2 | 90.2 (74.9–105.3) | 91 (75.5–105.4) | 90.1 (74.7–104.8) | 0.403 |
| Uric acid, mmol/L | 297.1 (225.9–375.8) | 296 (228.2–379.0) | 300 (221.1–365.0) | 0.426 |
| Magnesium, mmol/L | 0.83 ± 0.09 | 0.83 ± 0.09 | 0.81 ± 0.09 | 0.069 |
| Calcium, mmol/L | 2.2 (2.2–2.3) | 2.2 (2.2–2.3) | 2.2 (2.2–2.3) | 0.568 |
| Glucose, mmol/L | 6.7 (5.7–7.8) | 6.6 (5.6–7.7) | 7.1 (6.0–8.6) | 0.003 |
| PT, s | 13.5 (12.9–13.9) | 13.5 (13.0–13.9) | 13.4 (12.9–14.0) | 0.435 |
| Fibrinogen, g/L | 3.40 (2.80–3.90) | 3.40 (2.81–3.80) | 3.30 (2.80–3.90) | 0.571 |
| APTT, s | 34.9 (32.4–37.1) | 34.9 (32.5–37.0) | 34.9 (31.8–37.1) | 0.917 |
| TT, s | 16.5 (15.9–17) | 16.5 (15.9–17) | 16.5 (15.9–16.9) | 0.950 |
| INR | 1 (1.0–1.1) | 1 (1.0–1.1) | 1 (1.0–1.1) | 0.259 |
| CRP, mg/L | 5.3 (1.7–11.7) | 5.2 (1.7–11.7) | 5.9 (1.6–11.7) | 0.609 |
| Pre-ICH mRS | 0 (0–0) | 0 (0–0) | 0 (0–0) | 0.209 |
| NIHSS on admission | 11 (7–18) | 11 (6–16) | 14 (10−22) | <0.001 |
| GCS on admission | 13 (10–15) | 13 (11–15) | 13 (9–15) | 0.005 |

ICH indicates intracerebral hemorrhage; IVH, Intraventricular hemorrhage; SBP, systolic blood pressure; DBP, diastolic blood pressure; WBC, White blood cell; RBC, Red blood cell; ALT, Alanine transaminase; AST, Aspartate Aminotransferase; HDL, High density lipoprotein; LDL, Low density lipoprotein; LDH, lactate dehydrogenase; GFR, Glomerular filtration rate; PT, prothrombin time; APTT, activated partial thromboplastin time; TT, thrombin time; INR, international normalized ratio; CRP, C-reactive protein; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; GCS, Glasgow Coma Scale.

marginally significant in the multivariate analysis (Supplementary Table S5), which is consistent with previous reports to some extent.

A series of prior clinical studies gave strong clues regarding the role of DBil in HE. The association between DBil and stroke severity as well as poststroke outcomes has been investigated in several studies [22-26]. In particular, in a few studies that examined the relationship between DBil and ICH, Fu K et al. demonstrated the potential predictive ability of DBil for initial stroke severity and prognosis; namely, higher DBil levels were associated with greater stroke severity at presentation and worse outcomes at discharge [27]. In our study, we found that DBil was associated with HE and could predict the risk of HE in a linear relationship. Our results indicated that even if the DBil level is within the normal range, a level of 1.0 μmol/L may increase the risk of HE by approximately 8%, and a DBil level higher than 5.65 μmol/L increased the risk by four- to five fold. Direct bilirubin, as the end metabolic product that comprehensively reflects the multiple pathological pathways, metabolizes rapidly and can timely reflect the state of the body, which is a sensitive warning indicator. Our findings expand our understanding of the role of DBil in ICH, especially in the early prediction of HE.

The metabolic process and pathological effect of DBil per se may also support its potential predictive role in HE. There is a redox reaction cycle between biliverdin and bilirubin in the metabolism of bilirubin. In this case, bilirubin plays an antioxidant role, that is, bilirubin is traditionally regarded as an antioxidant factor [28]. Acute ICH will stimulate the body to produce an overall inflammatory response, and the production of a large number of oxygen radicals will induce the increase of bilirubin to play the antioxidant role. However, the antioxidant effect of bilirubin is limited to the state of low content [28], and more bilirubin will

**Table 2**
Multivariate analysis assessing the association between laboratory indicators and hematoma expansion.

| | Model 1[a] | Model 2[b] |
|---|---|---|
| | P Value OR (95%CI) | P Value OR (95%CI) |
| Presence of IVH | 0.022 1.553 (1.066–2.261) | 0.026 1.535 (1.052–2.240) |
| Time from symptom onset to first CT scan | 0.001 0.867 (0.797–0.944) | 0.001 0.866 (0.795–0.943) |
| Direct bilirubin | 0.023 1.082 (1.011–1.158) | 0.048 1.072 (1.001–1.149) |
| NIHSS on admission | 0.003 1.028 (1.010–1.046) | 0.004 1.027 (1.008–1.045) |
| INR | – | 0.053 3.934 (0.973–15.908) |

IVH indicates intraventricular hemorrhage; NIHSS, National Institutes of Health Stroke Scale; ICH, intracerebral hemorrhage; SBP, systolic blood pressure; LDH, lactate dehydrogenase; INR, international normalized ratio; CRP, C-reactive protein; GCS, Glasgow Coma Scale; OR, odds ratio; CI, confidence interval.
[a] Model 1 including age, sex and variables with P values<0.1 from the univariate analysis (Subarachnoid space extension, Presence of IVH, Time from symptom onset to first CT scan, Neutrophil percentage, Platelet count, Direct bilirubin, Magnesium, Glucose, NIHSS on admission).
[b] Model 2 including variables in Model 1 and variables previously shown to be predictive of HE (Oral anticoagulation, Admission SBP, Albumin, LDH, Calcium, Fibrinogen, INR, CRP, GCS on admission).

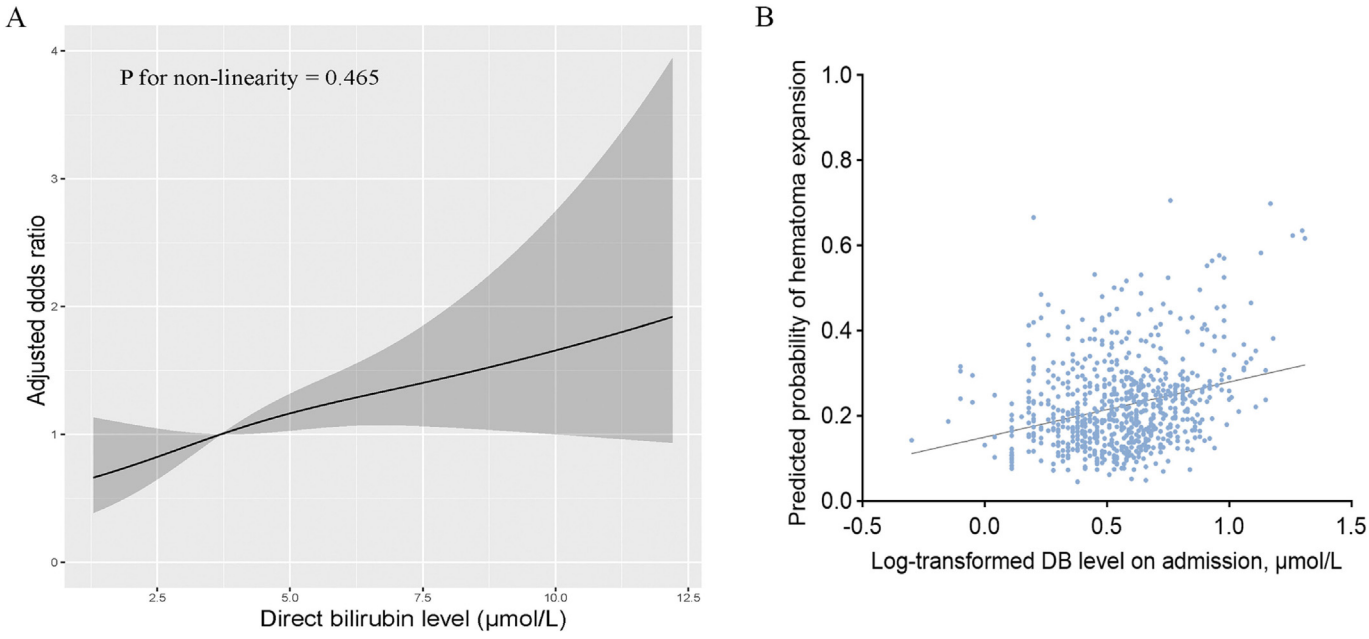


**Fig. 2.** A. Relationship of direct bilirubin level with the risk of hematoma expansion. Adjusted for age, sex, time from symptom onset to first CT scan, subarachnoid space extension, presence of IVH, PLT, glucose, magnesium, neutrophil percentage, NIHSS on admission, GCS on admission. B. Relationship between direct bilirubin level and predicted probability of hematoma expansion.

produce oxidative stress and other harmful effects. Bilirubin in the blood can reach the brain through the blood-brain barrier(BBB) and cause irreversible brain damage through pathological processes such as oxidative stress and activation of cytokines (including MMPs) [29-32]. Oxidative stress can destroy tight-junction proteins, degrade collagen and laminin in the basal membrane [33], and the activation of MMP-2 and MMP-9 can damage the integrity of cerebral microvascular endothelial cells, thus destroying the integrity of the BBB [34]. In addition, in the early stage of ICH, red blood cells in hematoma can be lysed [5], thereby releasing hemoglobin and further degrading to heme, which is transported to microglia, macrophages, vascular smooth muscle cells, neurons and endothelial cells and metabolized into bilirubin. Oxidative products are produced in these process and cause oxidative stress [35] which can promote inflammation, endoplasmic reticulum

**Table 3**
Multivariate analysis assessing association of direct bilirubin levels with hematoma expansion in validation cohort.

| | Model 1[a] | Model 2[b] |
|---|---|---|
| | *P* Value OR (95%CI) | P Value OR (95%CI) |
| Age, y | 0.233 1.030 (0.987–1.077) | 0.185 1.037 (0.998–1.082) |
| Male sex | 0.189 2.505 (0.739–9.774) | 0.163 1.959 (0.600–6.733) |
| Direct bilirubin >5.65 μmol/L | 0.011 4.476 (1.404–14.270) | 0.010 5.088 (1.523–18.964) |

OR indicates odds ratio; CI, confidence interval.
[a] Adjusted for age, sex, time from symptom onset to first CT scan, diabetes mellitus, previous stroke, initial ICH volume, deep ICH, NIHSS on admission.
[b] Adjusted for covariates from model 1 and further adjusted for admission SBP, GCS on admission.

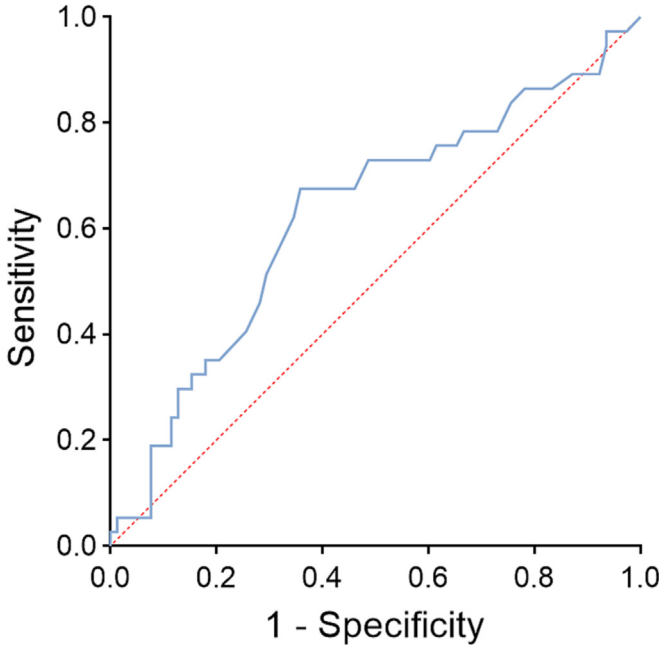


**Fig. 3.** Receiver operating characteristic (ROC) curves for prediction of direct bilirubin level for hematoma expansion in the validation cohort. The ROC curve yielded an area under the curve (AUC) value of 0.656 (95% CI 0.535–0.776, $P = 0.013$).

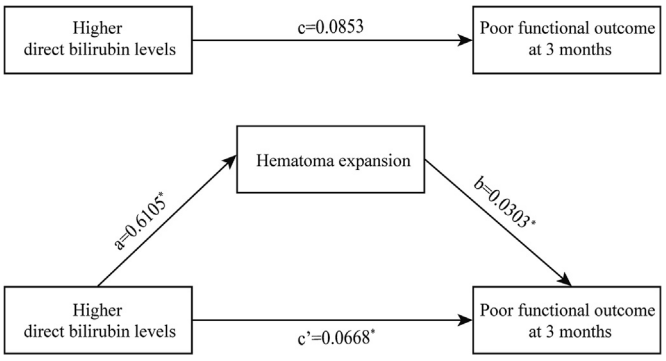


**Fig. 4.** The mediation analysis among direct bilirubin, hematoma expansion and 3-month functional outcome. Direct bilirubin was the independent variable, hematoma expansion was the intermediate variable, and 3-month functional outcome was the dependent variable. The total effect c of the independent variable on the dependent variable was 0.0853, the direct effect c' was 0.0668, and the indirect effect a*b was 0.0185. The 95% confidence interval of the indirect effect and direct effect did not include 0, so hematoma expansion played a partial mediating role, accounting for 21.69% of the whole effect.

stress, autophagy, apoptosis and necrosis, leading to the endothelial cell damage and destruction of BBB [36]. In addition, previous studies have found that elevated bilirubin concentration can inhibit platelet function, coagulation pathways and hemostatic response [37]. The effects of bilirubin on promoting inflammation, damaging vascular integrity, inhibiting coagulation and hemostasis provided evidence for motivating HE. During the above pathological reactions, bilirubin is supposed to act and cooperate with the reported biomarkers of HE, such as c-Fn [10], MMP-9 [13,38], IL-6 [7,10], CRP [7] and magnesium [15,39], and these associations are not limited to ICH [40-44]. Indeed, the pathological mechanism of HE is complex, and these pathological processes are likely to be related to each other [45-47].

This study had several limitations. First, it was a single-center study, which can lead to selection bias. However, Tongji Hospital is a national tertiary hospital and has wide coverage and a large number of patients. In addition, internal validation was performed to increase the consistency of the results. It is expected that our observations will be validated at other institutions. Second, the bilirubin data used in our study were collected within 24 h after system onset, and variations may have occurred compared with the initial condition. To guarantee reliability, we limited this time to 6 h in the validation cohorts. Third, data on the history of digestive diseases, which may affect blood bilirubin levels, were incomplete in our retrospective analysis. Nevertheless, the intergroup comparisons of liver function parameters on admission showed no significant differences, as did the history of chronic liver disease in the validation cohorts.

In conclusion, DBil is associated with HE after ICH and has predictive value for HE. DBil also acts as a predictor of poor post-ICH outcomes, and the relationship between DBil and outcomes was partially mediated by HE. Weather the decrement of DBil level takes effect in the development of HE warrants further prospective studies. Additional research is needed to explore the potential treatment interventions targeting DBil to improve prognosis after ICH.

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## CRediT authorship contribution statement

**Yuchao Jia:** Writing – original draft, Methodology, Investigation, Conceptualization. **Xiaodong Ye:** Investigation. **Guini Song:** Investigation. **Xianxian Li:** Investigation. **Jiahe Ye:** Investigation. **Yuyan Yang:** Investigation. **Kai Lu:** Investigation. **Shanshan Huang:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Suiqiang Zhu:** Supervision, Project administration, Funding acquisition.

## Data availability

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

## Declaration of Competing Interest

None.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajem.2023.06.042.

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