Translational Stroke Research (2019) 10:620–629
https://doi.org/10.1007/s12975-019-0686-7

ORIGINAL ARTICLE

Lactate Dehydrogenase Predicts Early Hematoma Expansion and Poor Outcomes in Intracerebral Hemorrhage Patients

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Received: 26 July 2018 / Revised: 26 December 2018 / Accepted: 2 January 2019 / Published online: 31 January 2019

Abstract

This study aimed to investigate whether serum lactate dehydrogenase (LDH) levels predicted hematoma expansion and poor outcomes in intracerebral hemorrhage (ICH) patients. The differentially expressed proteins between patients with and without hematoma expansion were screened using proteomic analysis. Then the critical value of the target protein was determined by retrospectively analyzing the data from a derivation cohort. A prospective study on the validation cohort of three clinical centers was performed to investigate the association between the target protein and hematoma expansion and poor outcomes (modified Rankin Scale > 3) at 90 days by using univariate and multivariate logistic regression analyses. Among the 41 differentially expressed proteins, LDH A chain was upregulated, which is one of the two main subunits of LDH protein. Considering that it was easy to determine serum LDH levels, LDH was selected as the target protein. In the derivation cohort, LDH ≥ 220 U/L was selected as the critical value to predict hematoma expansion by using receiver operating characteristic analysis. A total of 366 ICH patients were enrolled in the validation cohort and LDH ≥ 220 U/L was positive in 127 patients (34.7%). The multivariate logistic regression analysis demonstrated LDH levels and LDH ≥ 220 U/L independently predicted hematoma expansion ($p < 0.001$) and poor outcomes ($p < 0.001$). The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of LDH ≥ 220 U/L for hematoma expansion and poor outcome prediction were 79.1%, 80.0%, 56.7%, 92.1%, and 79.8% and 53.3%, 78.2%, 63.0%, 70.7%, and 68.0%, respectively. In conclusion, LDH is a reliable predictor for early hematoma expansion and poor outcomes in patients with ICH.

Keywords Intracerebral hemorrhage · Hematoma expansion · Lactate dehydrogenase · Proteomic analysis · Poor outcomes

Introduction

Intracerebral hemorrhage (ICH) is the second most common cause of cerebrovascular diseases, while it is the most lethal type and accounts for approximately 15% of all strokes worldwide [1, 2]. ICH has a high mortality and morbidity with poor outcomes. However, the treatment of ICH is still limited despite the unremitting efforts for exploring the effective managements. In general, early intensive intervention is very crucial for ICH treatment [3]. Hematoma expansion, which is often observed at the early stage of ICH, independently predicts poor outcomes including death and disability [4, 5]. Attenuation of the expansion is an intriguing therapeutic strategy for clinical benefits. Therefore, early rapid identification of the risk of hematoma expansion becomes the crux.

Signs from radiological examination such as computed tomography angiography (CTA) spot sign and blend sign and black hole sign by noncontrast CT (NCCT) are able to predict hematoma expansion and poor outcomes [6, 7]. However,

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these signs should be manually identified, which may lead to bias. Therefore, the predictors from laboratory tests may be more objective and of convenient use. To date, the laboratory parameters are mainly concentrated on the aspects of coagulation function, inflammation, and microvascular integrity. For example, serum calcium, C-reactive protein (CRP), and cellular fibronectin are regarded to predict early hematoma expansion and/or poor outcomes [8–10].

Based on the above theory, we hypothesized that one or more proteins may be more appropriate to predict hematoma expansion and poor outcomes. Here, such proteins were first identified from the differentially expressed proteins between the patients with or without hematoma expansion according to proteomic analysis. Then, the critical value was retrospectively explored through a derivation cohort, and whether the serum protein was an appropriate candidate for hematoma expansion and poor outcome prediction was subsequently prospectively investigated via the data from a validation cohort.

Materials and Methods

Study Population

A consecutive series of patients with primary ICH aged 18 years or more from Huashan Hospital, Fudan University (Center 1) between May 1, 2014 and December 31, 2014 were enrolled for proteomic analysis ($n = 52$). The enrolled patients all underwent baseline NCCT scan within 6 h after ICH onset and the follow-up NCCT scan was performed within 24 h after initial CT scan. The exclusion criteria included (1) secondary ICH (cerebral aneurysm, Moyamoya syndrome, arteriovenous malformation, tumor, trauma, or hemorrhagic transformation from brain infarction), (2) undergoing surgical evacuation before follow-up NCCT scan, (3) primary intraventricular hemorrhage (IVH), (4) a baseline ICH volume of less than 1 mL, and (5) historical modified Rankin Scale (mRS) scores > 1. The differentially expressed proteins were screened using proteomic analysis in this section for further study. The target protein should be featured by low cost, rapid detection, and availability in almost every emergency room.

Derivation cohort: A consecutive series of patients with primary ICH from Huashan Hospital, Fudan University (Center 1) between January 1, 2010 and April 30, 2014 were enrolled for retrospective analysis ($n = 162$). The inclusion and exclusion criteria were the same as above except the additional inclusion criteria that serum lactate dehydrogenase (LDH) levels were measured within 6 h after ICH onset. The critical value of LDH was determined via retrospective analysis on the data of this cohort.

Validation cohort: A consecutive series of patients with primary ICH admitted from three clinical centers of Fudan University: Huashan Hospital (Center 1), Zhongshan Hospital (Center 2), and Jinshan Hospital (Center 3) between January 1, 2015 and March 31, 2018, were enrolled for prospective analysis ($n = 366$). The inclusion and exclusion criteria were the same as those in the derivation cohort. Whether LDH predicted hematoma expansion and poor outcomes in ICH patients of this cohort was prospectively investigated. All patients or their next-of-kin gave their informed consent prior to inclusion in this study. This study was approved by and studied in accordance with the ethical standards of Fudan University Ethics Committee.

Imaging Analysis

NCCT examinations were performed using a multidetector CT scanner with contiguous axial 5-mm section thickness (Brilliance iCT; Philips Medical Systems, Cleveland, OH, USA). The hematoma was three-dimensionally reconstructed and the volume was measured volume by 3D Slicer software, which is a free open-source software platform for biomedical research (http://www.slicer.org). CT image data sets were acquired in the DICOM format and hematomas were automatically identified pixel by pixel in each slice after setting the threshold range at 50–100 HU. A 3D model was constructed and the hematoma volume was calculated by the accumulating volume of the pixels [11]. Hematoma expansion was defined as an absolute growth greater than 6 mL or a relative growth of more than 33% in the follow-up NCCT scan (within 24 h after initial CT scan) compared with the baseline NCCT scan (within 6 h after ICH onset) [12].

Proteomic Analysis

The blood samples were taken from the patients with or without hematoma expansion within 6 h after ICH onset, which were then stabilized with EDTA and immediately placed on ice, followed by centrifugation at 1500*g* for 10 min at 4 °C. The plasma samples were stored at − 80 °C for proteomic analysis. Processed proteins (100 μg) from each sample solution were digested with trypsin solution at 37 °C for 12 h. Liquid chromatography and tandem mass spectrometry (LC-MS/MS) were performed on a LC-20AB HPLC system (Shimadzu, Japan). Eluted peptides were collected into 20 fractions. Supernatant peptides were loaded onto a LC-20 AD nanoHPLC (Shimadzu, Japan) using an autosampler and a 2-cm C18 trap column. Then, peptides were eluted onto a 15-cm analytical C18 column, which was packed inhouse. The injection volume was 10 μL, and the flow rate was 300 nL/min. Peptide acquisition was performed with a Triple TOF 5600 System (AB Sciex, USA) fitted with a Nanospray III source (AB Sciex) and a pulled quartz tip as the emitter (New Objectives, USA). MS/MS scans were performed in high-sensitivity mode. For

peptide identification, all the spectra were analyzed using Proteome Discoverer (version 1.4.0.29, Thermo Fisher Scientific) using SEQUEST-HT (Thermo Fisher Scientific). For database searching, the false discovery rate was calculated with inverted databases and the refined method [13]. The differentially expressed proteins between the groups of hematoma expansion and nonexpansion were defined as $p < 0.05$ by t test and > 1.5-fold change.

Clinical Data

The essential clinical data, including sex, age, history of hypertension, diabetes mellitus, smoking, alcohol consumption, antiplatelet therapy and warfarin use, systolic and diastolic blood pressure at admission, serum LDH levels, time of ICH onset, and Glasgow Coma Scale (GCS) scores, were recorded after admission. The time of initial CT, location of the hematoma, presence of IVH, and hematoma volume after the initial NCCT scan were also recorded.

The functional outcome was assessed by using mRS at 90 days after ICH onset in the validation cohort. It was performed through in-person interviews by trained senior physicians or a phone call by trained staffs. Poor outcomes were defined as mRS > 3 and good outcomes was defined as mRS ≤ 3.

Statistical Analysis

All data were analyzed with SPSS22.0. Data for categorical variables were expressed as a percentage and compared using χ^2 test or Fisher exact test (two-tailed). Data for continuous variables were presented as means (standard deviations [SDs]) or medians (interquartile ranges [IQRs]) when appropriate and analyzed using two-tailed Student's t test, one-way analysis of variance, Mann-Whitney U test, or Kruskal-Wallis H test depending on the data distribution and the number of variables. Differences were regarded statistically significant at a value of $p < 0.05$. The ROC analysis was performed to determine the critical value of LDH for predicting hematoma expansion. Univariate analysis was used for comparing the variables to discover the possible significant predictors for hematoma expansion and poor outcomes. When significance appeared, multivariate logistic regression analysis was performed to investigate independent predictors for hematoma expansion and poor outcomes. Meanwhile, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of LDH for hematoma expansion and poor outcome prediction were also calculated. All analyses were conducted by statisticians blinded to the groups.

Results

Results of Proteomic Analysis

Fifty two out of 98 ICH patients were included for proteomic analysis according to the inclusion and exclusion criteria (Fig. 1). Hematoma expansion was found in 12 patients (23.1%). The baseline clinical and radiological characteristics were shown in Table 1. There were no significant differences in the clinical and radiological variables between patient with and without hematoma expansion except baseline GCS

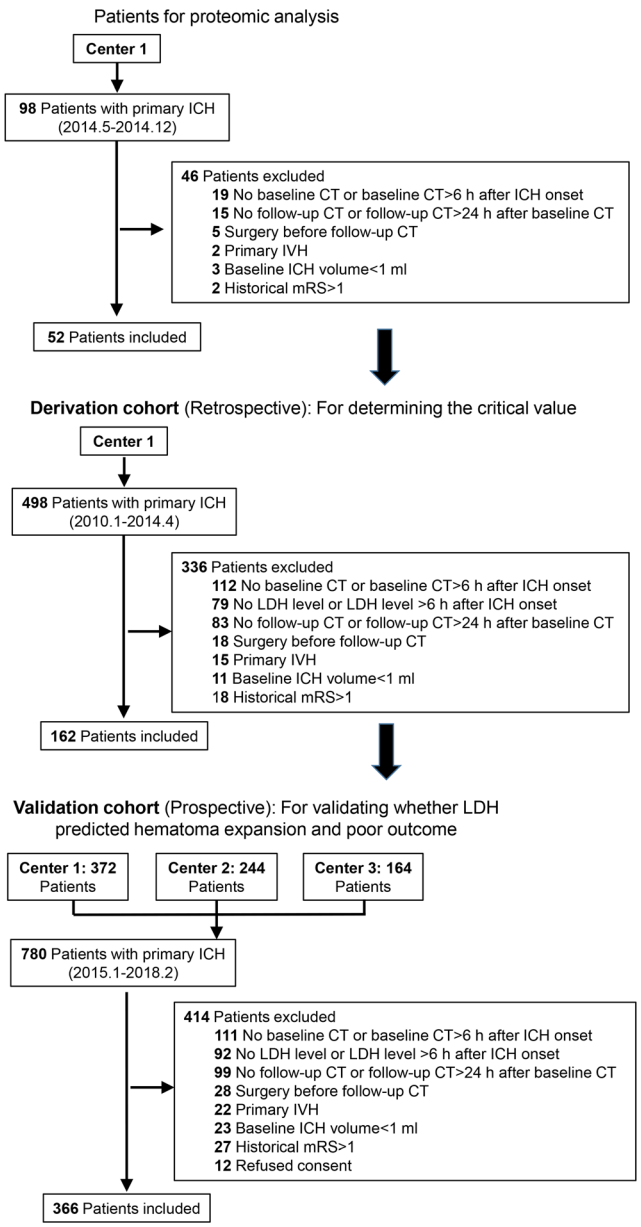

Fig. 1 Flowchart of study patients. CT computed tomography, ICH intracerebral hemorrhage, IVH intraventricular hemorrhage, LDH lactate dehydrogenase, mRS modified Rankin Scale

Table 1 Comparison of baseline clinical and radiological characteristics of the three cohorts

Characteristics	Patients for proteomic analysis ($n = 52$)	Derivation cohort ($n = 162$)	Validation cohort ($n = 366$)	p Value
Age (years), mean (SD)	62.6 (11.8)	64.3 (12.1)	63.6 (12.2)	0.824
Sex, male (%)	35 (67.3)	112 (69.1)	253 (69.1)	0.964
Hypertension (%)	36 (69.2)	111 (68.5)	252 (68.9)	0.995
Diabetes mellitus (%)	10 (19.2)	25 (15.4)	53 (14.5)	0.667
Smoking (%)	10 (19.2)	28 (17.3)	84 (23.0)	0.319
Alcohol consumption (%)	6 (11.5)	14 (8.6)	52 (14.2)	0.198
Antiplatelet therapy (%)	8 (15.4)	24 (14.8)	63 (17.2)	0.774
Warfarin use (%)	4 (7.7)	14 (8.6)	35 (9.6)	0.879
SBP on admission (mm Hg), mean (SD)	164.5 (29.2)	162.2 (27.9)	162.3 (28.3)	0.764
DBP on admission (mm Hg), mean (SD)	94.6 (17.5)	92.7 (15.5)	92.8 (15.9)	0.547
Baseline GCS score, median (IQR)	14 (12–15)	14 (12–15)	14 (11–15)	0.735
Time to the baseline NCCT (min), median (IQR)	160.5 (103.0–281.0)	154.5 (99.5–276.0)	143.0 (88.0–239.0)	0.476
Hematoma location				
Deep (%)	35 (67.3)	112 (69.1)	254 (69.4)	0.954
Lobar (%)	14 (26.9)	38 (23.5)	83 (22.7)	0.793
Infratentorial (%)	3 (5.8)	12 (7.4)	29 (7.9)	0.856
IVH (%)	13 (25.0)	34 (21.0)	132 (36.1)	0.002
Baseline hematoma volume (mL), median (IQR)	8.8 (4.5–17.8)	9.6 (4.8–19.2)	9.7 (5.0–22.0)	0.756
Hematoma expansion (%)	12 (23.1)	36 (22.2)	91 (24.9)	0.797
LDH (U/L), mean (SD)	/	202 (48.5)	207.6 (53.2)	0.308

DBP diastolic blood pressure, GCS Glasgow Coma Scale, IQR interquartile range, IVH intraventricular hemorrhage, LDH lactate dehydrogenase, NCCT noncontrast computed tomography, SBP systolic blood pressure, SD standard deviation

scores, time to the baseline NCCT, and baseline hematoma volume.

A total of 41 differentially expressed proteins were detected: 15 were upregulated and 26 downregulated (Fig. 2 and Table 2). It was demonstrated that LDH A chain (3.85-fold change, $p = 0.005$) was upregulated, which is one of the main chains of LDH protein. Therefore, it was speculated that LDH was significantly increased after hematoma expansion compared with nonexpansion. Moreover, it was easy to determine serum LDH levels, and determination of serum LDH levels was performed in almost every ICH patient. As a result, we selected LDH for subsequent study.

Baseline Characteristics of the Derivation and Validation Cohorts

After application of the eligibility criteria, 162 of 498 patients with primary ICH in the derivation cohort and 366 of 780 patients with primary ICH in the validation cohort were enrolled for analysis (Fig. 1).

In the derivation cohort, the median baseline GCS score was 14 (IQR 12–15). The median of time to the baseline NCCT scan was 154.5 min (IQR 99.5–276.0) from ICH onset with the baseline hematoma volume of 9.6 (IQR 4.8–19.2). The average of the LDH level was 202 ± 48.5 (U/L) (Table 1).

A total of 366 ICH patients (253 males and 113 females) were included in the validation cohort with the average age of (63.6 ± 12.2) years. There were 63 (17.2%) and 35 (9.6%) patients receiving antiplatelet therapy and warfarin therapy, respectively. The baseline GCS score was 14 (IQR 11–15). The median of time to the baseline NCCT scan was 143.0 min (IQR 88.0–239.0) from ICH onset with the baseline hematoma volume of 9.7 (IQR 5.0–22.0). The location of the baseline hematoma contained deep (69.4%), lobar (22.7%), and infratentorial (7.9%) and IVH was found in 36.1% of the patients. The average LDH level was 207.6 ± 53.2 (U/L). Hematoma expansion was observed in 91 patients (24.9%). One-hundred fifty (41.0%) patients had poor outcomes (mRS > 3), and the median mRS was 3 (IQR 2–4) (Table 1).

The Critical Value of LDH Levels Determined by ROC Analysis

To determine the critical value of LDH levels, we resorted to ROC analysis in the derivation cohort. The area under the curve (AUC) was 0.826 with 95% confidence interval (CI) of 0.748–0.904. The LDH level of 220 U/L paralleled with the optimal sensitivity, specificity, and Youden index (sensitivity + specificity − 1) of 75.0%, 81.0%, and 0.560, respectively, for predicting hematoma expansion (Fig. 3a). As a

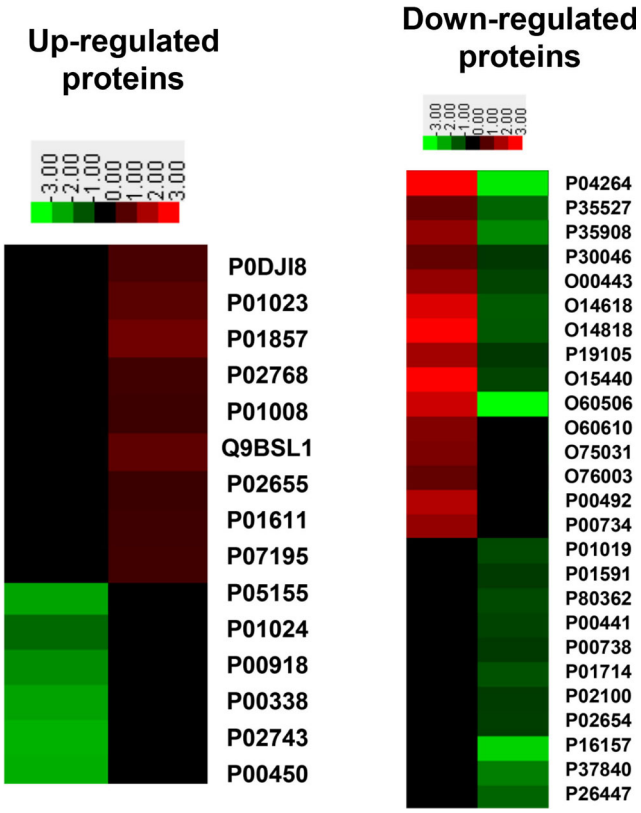

Fig. 2 Cluster analysis of the changes in abundance observed in the differentially expressed proteins

result, we further validated whether LDH ≥ 220 U/L was independently associated with hematoma expansion and poor outcomes was further validated.

LDH as a Reliable Predictor of Hematoma Expansion

Of all the clinical and radiological variables, baseline GCS scores, IVH, baseline hematoma volume, hematoma expansion, mRS scores, and poor outcomes were significantly different among different levels of LDH ($p < 0.05$) (Table 3). In order to enhance the clinical value, the critical value was harvested in the derivation cohort. LDH ≥ 220 U/L was found in 127 patients (34.7%) and observed in 72 patients with hematoma expansion (56.7%) (Table 3). For hematoma expansion prediction, univariate analysis revealed significant differences in warfarin use ($p < 0.001$), baseline GCS scores ($p < 0.001$), time to the baseline NCCT ($p < 0.001$), baseline hematoma volume ($p = 0.001$), LDH level ($p < 0.001$), and LDH ≥ 220 U/L ($p < 0.001$) (Table 4). When performing the multivariate logistic regression analysis, the sample size was not changed and still remained as 366 because patients with missing clinical or neurological data were excluded. Since sufficient sample size was ensured, the multiple comparisons were adequately controlled. The multivariate logistic regression analysis showed that LDH levels [odds ratio (OR) 1.032, 95% CI 1.022–1.041, $p < 0.001$] and LDH ≥ 220 U/L (OR 21.554, 95% CI 8.484–54.725, $p < 0.001$) were independently associated with hematoma expansion. Other predictors included warfarin use ($p = 0.023$) and time to the baseline NCCT ($p < 0.001$) (Table 5). The sensitivity, specificity, PPV, NPV, and accuracy of LDH ≥ 220 U/L for hematoma expansion prediction were 79.1%, 80.0%, 56.7%, 92.1%, and 79.8%, respectively. In ROC curve, the AUC was 0.796 with the 95% CI of 0.740–0.851 (Fig. 3b).

Association of LDH and Poor Outcomes

LDH ≥ 220 U/L was found in 80 patients with poor outcomes (63.0%). mRS scores in patients with LDH ≥ 220 U/L and LDH < 220 U/L were also detailed (Fig. 4). There were significant differences in warfarin use ($p = 0.019$), baseline GCS scores ($p < 0.001$), baseline hematoma volume ($p < 0.001$), LDH levels ($p < 0.001$), and LDH ≥ 220 U/L ($p < 0.001$) revealed by univariate analysis (Table 4). Subsequently, the multivariate logistic regression analysis demonstrated that baseline hematoma volume ($p < 0.001$), LDH levels (OR 1.025, 95% CI 1.016–1.033, $p < 0.001$), and LDH ≥ 220 U/L (OR 10.227, 95% CI 4.156–25.164, $p < 0.001$) were independent predictors for poor outcomes (Table 5). Moreover, the sensitivity, specificity, PPV, NPV, and accuracy of LDH ≥ 220 U/L for predicting poor outcomes were 53.3%, 78.2%, 63.0%, 70.7%, and 68.0%, respectively. The AUC of ROC curve was 0.658 with the 95% CI of 0.600–0.716 (Fig. 3c).

Discussion

Since hematoma expansion mainly occurs within the first 6 h after ICH onset, early identification and prevention are critical for acute ICH treatment [14]. The parameters from laboratory tests are relatively objective and easy to identify compared with neuroradiological findings, which increases the advantages in predicting hematoma expansion. There were three cohorts in our study. There were no significant differences among the three cohorts in the baseline characteristics except IVH, which may be attributed to a higher proportion in Center 2 and Center 3. The differentially expressed proteins were first screened from the blood samples of ICH patients with or without hematoma expansion using proteomic analysis. Among the positive proteins, LHD, complement C3, and ceruloplasmin are common in the clinical work. LDH is easily and rapidly detectable and available in almost every patient in the emergency room and ward with low costs. However, detection of complement C3 and ceruloplasmin is feasible only in the ward of the three centers and the testing results are unavailable until the next day. As a result, LDH was selected as the target protein. To augment the clinical application, we retrospectively explored the critical value of LDH for hematoma expansion prediction through ROC analysis according to

Table 2 The list of the differentially expressed proteins

Accession	Protein names	Fold change	*p* Value
Upregulated			
P0DJI8	Serum amyloid A-1 protein	1.82	0.024
P01023	Alpha-2-macroglobulin	2.08	0.034
P01857	Immunoglobulin heavy constant gamma 1	2.51	0.028
P02768	Serum albumin	1.73	0.025
P01008	Antithrombin-III	1.66	0.019
Q9BSL1	Ubiquitin-associated domain-containing protein 1	2.11	0.032
P02655	Apolipoprotein C-II	1.62	0.008
P01611	Immunoglobulin kappa variable 1D-12	1.68	0.041
P07195	L-lactate dehydrogenase B chain	1.71	0.018
P05155	Plasma protease C1 inhibitor	3.75	< 0.001
P01024	Complement C3	2.43	0.035
P00918	Carbonic anhydrase 2	3.60	0.016
P00338	L-lactate dehydrogenase A chain	3.85	0.005
P02743	Serum amyloid P-component	4.35	0.017
P00450	Ceruloplasmin	4.67	0.006
Downregulated			
P04264	Keratin, type II cytoskeletal 1	0.04	0.022
P35527	Keratin, type I cytoskeletal 9	0.22	0.014
P35908	Keratin, type II cytoskeletal 2 epidermal	0.12	0.023
P30046	D-dopachrome decarboxylase	0.31	< 0.001
O00443	Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit alpha	0.22	0.003
O14618	Copper chaperone for superoxide dismutase	0.10	0.041
O14818	Proteasome subunit alpha type-7	0.08	0.036
P19105	Myosin regulatory light chain 12A	0.21	0.009
O15440	Multidrug resistance-associated protein 5	0.08	0.017
O60506	Heterogeneous nuclear ribonucleoprotein Q	0.03	0.022
O60610	Protein diaphanous homolog 1	0.36	< 0.001
O75031	Heat shock factor 2-binding protein	0.41	0.016
O76003	Glutaredoxin-3	0.47	0.007
P00492	Hypoxanthine-guanine phosphoribosyltransferase	0.26	0.036
P00734	Prothrombin	0.32	0.008
P01019	Angiotensinogen	0.67	0.015
P01591	Immunoglobulin J chain	0.56	0.045
P80362	Ig kappa chain V-I region WAT	0.56	0.034
P00441	Superoxide dismutase [Cu-Zn]	0.59	0.005
P00738	Haptoglobin	0.56	0.012
P01714	Immunoglobulin lambda variable 3–19	0.54	0.001
P02100	Hemoglobin subunit epsilon	0.75	0.009
P02654	Apolipoprotein C-I	0.63	0.017
P16157	Ankyrin-1	0.23	0.038
P37840	Alpha-synuclein	0.36	< 0.001
P26447	Protein S100-A4	0.46	0.026

the data from derivation cohort. The sample size was further increased and a multicenter prospective study was performed in the validation cohort. It was demonstrated that both LDH levels and LDH ≥ 220 U/L could independently predict hematoma expansion ($p < 0.001$) with the sensitivity and specificity of 79.1% and 80.0%, respectively. Since hematoma expansion is associated with a poor prognosis [15], it should be more beneficial if the predictor for hematoma expansion is

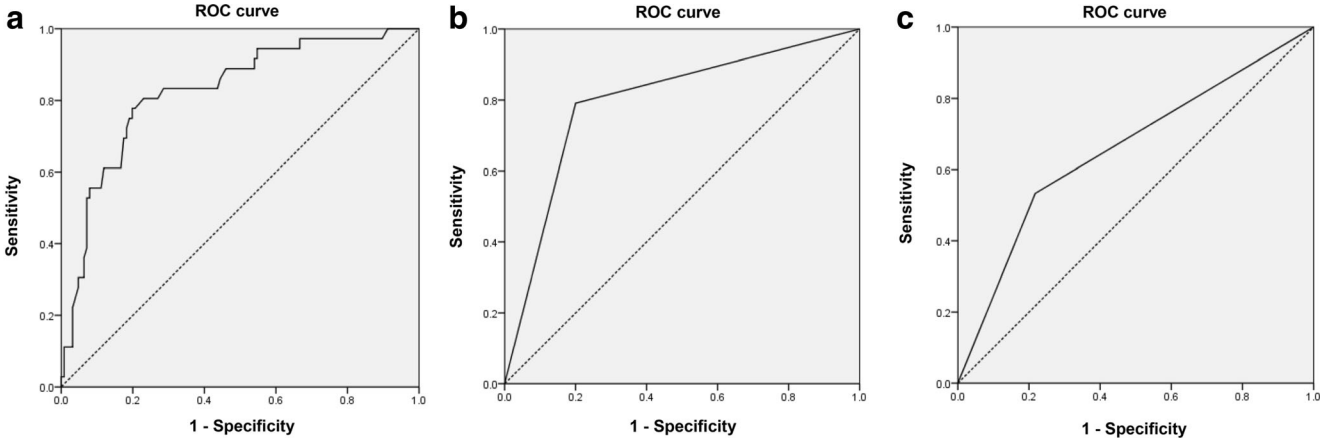

Fig. 3 Receiver operating characteristic (ROC) curves. **a** ROC analysis for determining the critical value of lactate dehydrogenase (LDH) level. **b** ROC curve of LDH ≥ 220 U/L for predicting hematoma expansion. **c** ROC curve of LDH ≥ 220 U/L for predicting poor outcome

also associated with poor outcomes. Correspondingly, we prospectively investigated whether LDH predicted poor outcome assessed by using mRS at 90 days after ICH in the validation cohort, and it was demonstrated that both LDH levels and LDH ≥ 220 U/L were independently associated with poor outcomes. Taken together, LDH can be regarded as a reliable predictor for hematoma expansion and poor outcomes.

Predicting hematoma expansion and/or outcomes in virtue of laboratory parameters is a hot topic. The mechanisms mainly focus on the following three aspects. Changes of coagulation status may increase the risk of bleeding and prevent hemostasis. It has been reported that low fibrinogen levels, international normalized ratio > 1.5, and hypocalcemia are associated with hematoma expansion [8, 16, 17]. Meanwhile, it is

Table 3 Comparison of the clinical and radiological characteristics between patients with different levels of LDH

Characteristics	LDH (U/L)				*p* Value
	≤ 149 (*n* = 43)	150–184 (*n* = 93)	185–219 (*n* = 103)	≥ 220 (*n* = 127)	
Age (years), mean (SD)	60.0 (14.5)	62.1 (11.3)	64.8 (11.2)	65.0 (12.5)	0.052
Sex, male (%)	31 (72.1)	70 (75.3)	68 (66.0)	86 (67.7)	0.498
Hypertension (%)	35 (81.4)	63 (67.7)	63 (61.2)	91 (71.7)	0.089
Diabetes mellitus (%)	8 (18.6)	12 (12.9)	14 (13.6)	19 (15.0)	0.833
Smoking (%)	12 (27.9)	21 (22.6)	21 (20.4)	30 (23.6)	0.797
Alcohol consumption (%)	8 (18.6)	13 (14.0)	12 (11.7)	19 (15.0)	0.730
Antiplatelet therapy (%)	6 (14.0)	14 (15.1)	17 (16.5)	26 (20.5)	0.658
Warfarin use (%)	3 (7.0)	6 (6.5)	9 (8.7)	17 (13.4)	0.308
SBP on admission (mm Hg), mean (SD)	156.0 (23.9)	162.6 (27.9)	161.7 (28.7)	164.7 (29.7)	0.397
DBP on admission (mm Hg), mean (SD)	92.5 (18.4)	94.9 (16.9)	90.2 (15.7)	93.4 (14.3)	0.230
Baseline GCS score, median (IQR)	14 (11–15)	15 (14–15)	14 (12–15)	13 (10–15)	0.001
Time to the baseline NCCT (min), median (IQR)	181.5 (94.5–300.5)	144.0 (95.5–234.5)	161.0 (99.5–256.5)	131.0 (80.0–218.0)	0.097
Hematoma location					
Deep (%)	34 (79.1)	67 (72.0)	67 (65.0)	86 (67.7)	0.349
Lobar (%)	4 (9.3)	18 (19.4)	27 (26.2)	34 (26.8)	0.074
Infratentorial (%)	5 (11.6)	8 (8.6)	9 (8.7)	7 (5.5)	0.578
IVH (%)	13 (30.2)	26 (28.0)	38 (36.9)	55(43.3)	0.102
Baseline hematoma volume (mL), median (IQR)	11.9 (3.9–23.3)	8.5 (4.2–12.5)	8.8 (4.9–20.8)	14.5 (6.5–28.0)	0.009
Hematoma expansion (%)	1 (2.3)	8 (8.6)	10 (9.7)	72 (56.7)	< 0.001
mRS scores, median (IQR)	3 (1–4)	2 (1–3)	2 (1–4)	4 (2–4)	< 0.001
Poor outcome, *n* (%)	13 (30.2)	20 (21.5)	37 (35.9)	80 (63.0)	< 0.001

DBP diastolic blood pressure, GCS Glasgow Coma Scale, IQR interquartile range, IVH intraventricular hemorrhage, LDH lactate dehydrogenase, mRS modified Rankin Scale, NCCT noncontrast computed tomography, SBP systolic blood pressure, SD standard deviation

Table 4 Univariate analysis of the potential predictors for hematoma expansion and poor outcome

Variables	Hematoma expansion ($n = 366$)			Poor outcome ($n = 366$)		
	Odds ratio	95% confidence interval	p Value	Odds ratio	95% confidence interval	p Value
Age (years)	0.996	0.977–1.016	0.701	1.002	0.985–1.019	0.850
Sex	1.292	0.760–2.197	0.345	1.275	0.806–2.016	0.299
Hypertension	1.358	0.799–2.307	0.258	1.218	0.774–1.918	0.393
Diabetes mellitus	1.231	0.642–2.361	0.532	0.853	0.469–1.553	0.603
Smoking	1.095	0.628–1.912	0.749	0.913	0.555–1.501	0.719
Alcohol consumption	1.009	0.512–1.987	0.980	0.661	0.355–1.230	0.191
Antiplatelet therapy	1.385	0.761–2.523	0.287	1.386	0.804–2.392	0.240
Warfarin use	3.742	1.836–7.626	< 0.001	2.349	1.153–4.784	0.019
SBP on admission (mm Hg)	1.004	0.995–1.012	0.362	1.006	0.998–1.014	0.124
DBP on admission (mm Hg)	1.002	0.987–1.018	0.767	1.011	0.997–1.025	0.130
Baseline GCS score	0.815	0.750–0.885	< 0.001	0.455	0.384–0.539	< 0.001
Time to the baseline NCCT (min)	0.992	0.989–0.996	< 0.001	0.999	0.997–1.002	0.635
Hematoma location						
Deep	0.990	0.592–1.654	0.968	0.805	0.514–1.262	0.345
Lobar	1.212	0.697–2.106	0.495	1.373	0.839–2.248	0.207
Infratentorial	0.608	0.225–1.643	0.327	0.432	0.179–1.038	0.061
IVH	1.381	0.850–2.243	0.193	1.333	0.865–2.054	0.192
Baseline hematoma volume (mL)	1.021	1.009–1.033	0.001	1.199	1.151–1.250	< 0.001
LDH (U/L)	1.027	1.021–1.034	< 0.001	1.016	1.011–1.021	< 0.001
LDH ≥ 220 U/L	15.158	8.440–27.223	< 0.001	4.109	2.606–6.480	< 0.001

DBP diastolic blood pressure, GCS Glasgow Coma Scale, IVH intraventricular hemorrhage, LDH lactate dehydrogenase, NCCT noncontrast computed tomography, SBP systolic blood pressure

well known that inflammatory responses play important roles in the primary and secondary neural injuries following ICH [18]. Therefore, increasing evidence supports that inflammation-related factors such as low neutrophil counts, high monocyte counts, CRP > 10 mg/L, and interleukin-6 > 24 pg/mL are able to predict hematoma expansion and/or poor outcomes [9, 10, 19]. In addition, endothelial damage leads to the breakdown of the blood-brain barrier and edema formation, which is closely related to functional deficits. Correspondingly, matrix metalloproteinase-9, cellular fibronectin, and low-density lipoprotein cholesterol that are markers of microvascular integrity are considered to be independently associated with hematoma expansion and/or poor outcomes [10, 20].

Table 5 Multivariate analysis of the predictors for hematoma expansion and poor outcome

Variables	Hematoma expansion ($n = 366$)			Poor outcome ($n = 366$)		
	Odds ratio	95% confidence interval	p Value	Odds ratio	95% confidence interval	p Value
Warfarin use	1.354	0.873–1.928	0.023	0.310	0.074–1.307	0.111
Baseline GCS score	0.812	0.631–1.044	0.104	0.856	0.639–1.147	0.297
Time to the baseline NCCT (min)	0.989	0.984–0.994	< 0.001	/	/	/
Baseline hematoma volume (mL)	0.983	0.948–1.019	0.344	1.209	1.120–1.304	< 0.001
LDH (U/L)	1.032	1.022–1.041	< 0.001	1.025	1.016–1.033	< 0.001
LDH ≥ 220 U/L	21.554	8.489–54.725	< 0.001	10.227	4.156–25.164	< 0.001

GCS Glasgow Coma Scale, LDH lactate dehydrogenase, NCCT noncontrast computed tomography

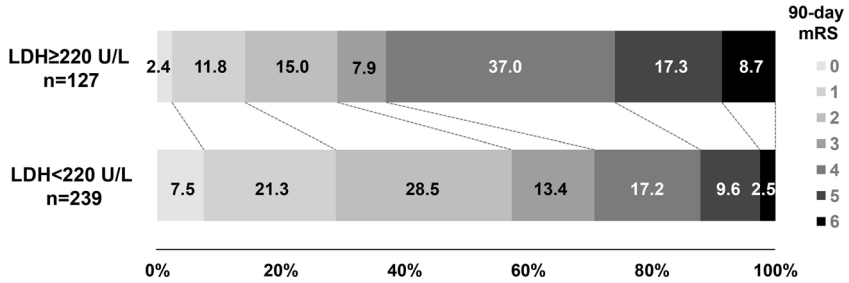

Fig. 4 The 90-day modified Rankin Scale (mRS) scores in patients with lactate dehydrogenase (LDH) ≥ 220 U/L and LDH < 220 U/L

LDH is an enzyme that is widely distributed in various cells and tissues, including muscle, liver, and brain. It is released into the peripheral blood after cellular damage. It has been reported that LDH levels are increased in patients with central nervous system (CNS) disorders such as cerebral infarction and hypoxic-ischemic encephalopathy [21, 22]. Meanwhile, according to Muiz's study, a high LDH level was a predictor of mortality in ICH patients [23]. The mechanism of LDH predicting hematoma expansion and poor outcomes in our research may be related to the association with inflammatory responses. LDH has been reported as a promising biomarker for inflammatory burdens, and its inhibitors can be used for antiinflammation [24, 25]. There are four LDH genes: *LDHA*, *LDHB*, *LDHC*, and *LDHD* and the first two genes code LDH A (M subunit) and LDH B (H subunit), forming five isoenzymes: LDH-1 (4H), LDH-2 (3H, 1M), LDH-3 (2H, 2M), LDH-4 (1H, 3M), and LDH-5 (5M). The serum LDH detected in clinic is comprised of the five isoenzymes [26]. Our proteomic analysis revealed that LDH A other than LDH B was upregulated after ICH, suggesting that the increased serum LDH mainly consists of LDH A. Moreover, LDH A is associated with vascular endothelial damage and angiogenesis [27], which may be another potential explanation of LDH to predict hematoma expansion and poor outcomes.

Since LDH was demonstrated as a predictor for hematoma expansion and poor outcomes, the potential clinical implications should be further investigated. Single predictor for hematoma expansion has its own limitation. Therefore, the prediction scores comprising of several predictors have been created [28, 29]. Correspondingly, LDH ≥ 220 U/L can be combined with other predictors to form a new prediction score with higher sensitivity and specificity. Moreover, LDH may also be applied to ICH treatment. It has been studied whether intensive blood pressure reduction improves outcomes of ICH patients with NCCT and CTA markers [30, 31]. Meanwhile, CTA spot sign is a key inclusion criterion of the trial to evaluate the effect of tranexamic acid [32]. Similarly, further studies may investigate whether ICH patients with LDH ≥ 220 U/L benefit from intensive blood pressure reduction or hemostasis therapy.

Several limitations of this study should be concerned. The sample size was relatively small. Meanwhile, although LDH ≥ 220 U/L in our work led to the optimized sensitivity, specificity, PPV, NPV, and accuracy, the critical value may be changed supposing the increase of sample size. Moreover, to simultaneously observe hematoma expansion, the patients undergoing surgical evacuation before follow-up NCCT scan and those without follow-up CT or follow-up CT > 24 h were excluded, which may reduce the sample size for predicting poor outcome. Besides, admission NIHSS was not used as a measure of ICH severity. Also, correlation of LDH ≥ 220 U/L with the CTA spot sign was not analyzed.

Conclusions

The current study using proteomic analysis and following investigation of derivation and validation cohorts suggests that serum LDH independently predicts hematoma expansion and poor outcomes. Meanwhile, our findings indicates that LDH ≥ 220 U/L can be regarded as a reliable predictor for early hematoma expansion and poor outcomes due to its feasibility and convenience for clinical practice.

Funding This research was supported by grants from the National Natural Science Foundation of China (No. 81500998) and Science and Technology Commission of Shanghai Municipality, China (No. 16140903200).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval and Patient Consent All procedures performed in studies involving human participants were in accordance with the ethical standards of the Fudan University Ethics Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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