Clinica Chimica Acta 492 (2019) 72–77

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Determination of serum neutrophil gelatinase-associated lipocalin as a prognostic biomarker of acute spontaneous intracerebral hemorrhage

Sheng Chen[a,*], Xia-Chan Chen[b], Xiao-Hui Lou[c], Song-Quan Qian[a], Zhan-Wei Ruan[d]

[a] Department of Emergency Medicine, Ruian People's Hospital, The Third Affiliated Hospital of Wenzhou Medical University, 108 Wansong Road, Ruian 325200, Zhejiang Province, China
[b] Department of Neurology, Ruian People's Hospital, The Third Affiliated Hospital of Wenzhou Medical University, 108 Wansong Road, Ruian 325200, Zhejiang Province, China
[c] Department of Neurosurgery, Ruian People's Hospital, The Third Affiliated Hospital of Wenzhou Medical University, 108 Wansong Road, Ruian 325200, Zhejiang Province, China
[d] Department of Emergency Intensive Care Unit, Ruian People's Hospital, The Third Affiliated Hospital of Wenzhou Medical University, 108 Wansong Road, Ruian 325200, Zhejiang Province, China

ARTICLE INFO

Keywords:
Intracerebral hemorrhage
Neutrophil gelatinase-associated lipocalin
Prognosis
Severity

ABSTRACT

Background: Neutrophil gelatinase-associated lipocalin (NGAL) is currently known as an acute phase protein and implicated in acute brain injury. Herein, we sought to gauge serum NGAL level in patients after acute (< 24 h) spontaneous intracerebral hemorrhage (ICH) and to investigate its relation to neurological outcome.
Methods: Serum NGAL levels were measured in 106 patients and 106 controls. National Institutes of Health Stroke Scale (NIHSS) score, Glasgow coma scale (GCS) score, ICH score and hematoma volume were recorded for assessing hemorrhagic severity. An unfavorable outcome was defined as modified Rankin Scale > 2 at 90 days.
Results: As opposed to the controls, the patients had significantly raised serum NGAL levels. Correlations were observed between NGAL levels and serum C-reactive protein levels, blood glucose levels, GCS score, NIHSS score, ICH score and ICH volume. Multivariate analysis identified serum NGAL as a predictor for unfavorable outcome at 90 days. It also showed high prognostic ability under receiver operating characteristic curve.
Conclusions: Enhanced NGAL level is revealed after acute spontaneous ICH, in association with inflammatory degree and hemorrhagic severity, and intimately correlated with a worse prognosis.

1. Introduction

Intracerebral hemorrhage (ICH) is the most disabling form of stroke [1–3]. Hematoma volume can greatly affect prognosis of ICH [4–6]. National Institutes of Health Stroke Scale (NIHSS) score can accurately assess clinical outcome of patients with ICH [7–9]. GCS score is often used to reflect the extent of consciousness in ICH patients [10–12]. ICH score is calculated based on five parameters, namely, age, hematoma volume, consciousness state, location of hematoma and presence of intraventricular extension [13–15]. To some extent, ICH score can be utilized to estimate hemorrhagic severity and predict prognosis of ICH [13–15]. Inflammatory response is an important process among all pathophysiological mechanisms underlying acute brain injury caused by intracerebral hematoma [16–18]. Upon the exposure of brain tissue to blood components, inflammatory cells in the injured brain, including neutrophils, monocytes and microglia, will be activated and a cascade of inflammatory reaction is triggered [16–18]. The accumulating evidence has shown that inflammation is strongly associated with an unfavorable outcome following hemorrhagic stroke [4,6,19]. Obviously, research regarding the role of inflammation in ICH has drawn a great attention in recent decades [16–19].

Neutrophil gelatinase-associated lipocalin (NGAL) is a member of the lipocalin family, which is formerly found to be highly expressed in neutrophils, kidney epithelial cells, alveolar macrophages, as well as bind or transport lipid and other hydrophobic molecules [20–22]. NGAL emerges as an acute phase protein and plays an important role in innate immune response. Because it could compete with bacteria for sequestering iron, NGAL was thought to defense bacterial invading [23,24]. Gradually, it was verified that expression of NGAL could be significantly up-regulated in animal brain tissues after hemorrhagic

Abbreviations: AUC, area under curve; CI, confidence interval; CT, computerized tomography; ICH, intracerebral hemorrhage; NIHSS, National Institutes of Health Stroke Scale; OR, odds ratio; ROC, receiver operating characteristic; GCS, Glasgow coma scale; NGAL, Neutrophil gelatinase-associated lipocalin

* Corresponding author.
E-mail address: xwbszf@126.com (S. Chen).

https://doi.org/10.1016/j.cca.2019.02.009
Received 14 January 2019; Received in revised form 2 February 2019; Accepted 12 February 2019
Available online 13 February 2019

injury. Moreover, its deleterious effects are mainly related to its enhancing iron toxicity and subsequently damaging brain tissues [25–28]. In a small number of humans with traumatic brain injury, raised serum NGAL levels were positively correlated with increasing severity [29]. Due to a paucity of data on circulating NGAL levels in ICH, the current study was designed to not only assess its relation to hemorrhagic severity, but also discern its prognostic ability in a group of patients with acute spontaneous ICH.

2. Materials and methods

2.1. Study population

In this prospective, observational study conducted at our hospital from January 2014 to March 2017. Our target group comprised consecutive patients with acute spontaneous ICH assessed in the emergency room within the first 24 h from stroke onset. According to inclusion criteria below: spontaneous cause for ICH, adult age (> 18 years old), and available serum NGAL level at admission, such ICH patients were initially evaluated. Exclusion criteria were as follows: the presence of underlying vascular lesions, traumatic hemorrhage, ICH resulting from venous sinus thrombosis, impaired coagulation, antiplatelet pretreatment, anticoagulant therapy, head trauma, hemorrhagic infarction, tumoral bleedings, acute or chronic infection(s) on admission or in the 4 weeks before ICH, and previous ischemic or hemorrhagic stroke and severe head trauma. The patients who underwent a surgical procedure and those who did not obtain follow-up computerized tomography (CT) scan of head were also excluded. Meanwhile, healthy individuals were enrolled as controls. The Institutional Ethics Committee approval for this study was granted and written informed consent was obtained from them or their relatives.

2.2. Baseline characteristics and outcome measures

On arrival to the emergency department, demographics (age, gender, body mass index), a detailed history of vascular risk factors (hypertension, diabetes mellitus, hyperlipidemia, congestive heart failure, current smoking, moderate-heavy alcohol consumption, coronary artery disease, chronic kidney disease and statin pretreatment), concomitant medication (antiplatelet pretreatment and oral anticoagulation pretreatment), blood pressure, body temperature and blood samples were taken, before the baseline head CT scan was performed. National Institutes of Health Stroke Scale (NIHSS) scores [7–9] and Glasgow coma scale (GCS) scores [10–12] were recorded to assess the neurological status and the level of consciousness on admission respectively, whereas baseline severity of ICH was quantified by ICH score [13–15]. All patients underwent CT scans of the head at hospital admission. Follow-up CT was acquired within 6 to 24 h of initial CT. Hematoma volume was measured based on ABC/2 method [30]. Hematoma expansion was defined as an absolute increase of > 12.5 mL or a relative increase of > 33% in hematoma volume at the 6- to 24-hour follow-up CT compared with the admission CT [31]. ICH location was categorized as deep or lobar and the presence of intraventricular or subarachnoidal extension of hematoma was also assessed at baseline and follow-up CT scans. Severe neurological dysfunction was referred to as NIHSS score > 10 points at admission [32], severe ICH was defined as ICH score > 2 points at admission [32], moderate - severe consciousness disturbance was considered when GCS score < 13 points [13], large hematoma volume was defined as hematoma volume > 30 mL [33] and early neurologic deterioration was defined as an increase of ≥4 points in the NIHSS score or death at 24 h from symptoms onset [34]. Modified Rankin Scale (mRS) score at 90 days were used to evaluate functional outcome [35]. Patients that scored > 2 points on the mRS were considered as having an unfavorable outcome.

2.3. Immunoassay methods

Blood samples for determination of NGAL levels were obtained from ICH patients within 24 h of stroke onset, similar to other laboratory values, such as glucose, platelets, C-reactive protein and lipid levels. Controls' blood samples were collected at study entry. Serum samples were separated from blood by centrifuging at 3000*g* for 10 min. Samples were stored at −80 °C until assayed. Every 3 months, NGAL levels were measured in duplicate samples using an enzyme-linked immunosorbent assay kit (R&D Systems), following the manufacturer's instructions. The results were expressed as ng/ml. The mean values of two measurements were used for further analyses. All determinations were performed by laboratory technicians blinded to all clinical data.

2.4. Statistical analysis

Statistical analyses were carried out using The Statistical Package for the Social Sciences version 19.0 (SPSS Inc., Chicago, IL, USA) and MedCalc 9.6.4.0 (MedCalc Software, Mariakerke, Belgium). Continuous variables with normal distributions were presented as means with standard deviation, while those with skewed distributions were presented as medians with interquartile range. Categorical variables were presented as percentages. Statistical comparisons between different subgroups were performed using the chi-square test, Fisher exact test, unpaired t-test, or Mann-Whitney *U* test as appropriate. To study correlations between continuous variables, Spearman correlation coefficients were used. Receiver operating characteristics curves were configured to establish the cut-off points of NGAL levels with the optimal sensitivity and specificity for predicting 90-day unfavorable outcome. Area under curve (AUC) and 95% confidence interval (CI) values were estimated. Univariable and multivariable logistic regression models assessed the associations of baseline characteristics with unfavorable outcome. In all univariable analyses, a threshold of $P < 0.1$ was used to identify candidate variables for inclusion in multivariable logistic regression model that tested statistical significance hypothesis using the likelihood ratio test with a value of 0.05. We reported all associations as odds ratios (ORs) in logistic regression models, with their corresponding 95% CIs. A *P* value of < 0.05 was considered significant for all test.

3. Results

3.1. Study population characteristics

A total of 106 ICH cases and 106 healthy controls were available for analysis. Forty-two patients with ICH were removed because of the preceding exclusion criteria. There were no significant differences in demographics between included and excluded ICH individuals to suggest significant bias from study selection criteria. There were significant differences for age, sex and body mass index between patients with ICH and controls. Among all patients (median age, 67 years [interquartile range, 58–77 years]; 57.6% men; mean body mass index, 26.2 ± 2.3 kg/m^2), Severe neurological dysfunction (NIHSS score > 10) was documented in 48 patients (45.3%), there were 8 patients (7.6%) with severe ICH (ICH score > 2), 26 patients (24.5%) experienced moderate-severe consciousness disturbance (GCS score < 13) and large hematoma volume (> 30 mL) appeared in 15 patients (14.2%). In total, 56 patients (52.8%) suffered from an unfavorable outcome. Other clinical, biochemical and radiological characteristics for the patients are presented in Table 1.

3.2. Serum NGAL levels

In Fig. 1, the patients showed substantially higher serum NGAL levels than the controls. Moreover, among those patients, there were a strong correlations between serum NGAL levels and NIHSS scores,

Table 1
Baseline clinical, biochemical and radiological characteristics and factors associated with 90-day unfavorable outcome in patients with acute spontaneous intracerebral hemorrhage.

Factors	All patients (n = 106)	Unfavorable outcome (mRS > 2)		
		Yes (n = 56)	No (n = 50)	P value
Gender (Male/Female)	61/45	32/24	29/21	0.929
Age (y)	67 (58–77)	74 (62–77)	66 (58–74)	0.023
Body mass index (kg/m^2)	26.2 ± 2.3	26.5 ± 2.3	25.8 ± 2.3	0.085
Current smoking	51 (48.1%)	27 (48.2%)	24 (48.0%)	0.982
Moderate-heavy alcohol consumption	42 (39.6%)	25 (44.6%)	17 (34.0%)	0.263
Hypertension	92 (86.8%)	48 (85.7%)	44 (88.0%)	0.729
Diabetes mellitus	32 (30.2%)	22 (39.3%)	10 (20.0%)	0.031
Hyperlipidemia	39 (36.8%)	20 (35.7%)	19 (38.0%)	0.808
Congestive heart failure	8 (7.6%)	6 (10.7%)	2 (4.0%)	0.191
Coronary artery disease	11 (10.4%)	7 (12.5%)	4 (8.0%)	0.448
Chronic kidney disease	9 (8.5%)	6 (10.7%)	3 (6.0%)	0.385
Statin pretreatment	29 (27.4%)	17 (30.4%)	12 (24.0%)	0.464
Lobar hemorrhage	26 (24.5%)	16 (28.6%)	10 (20.0%)	0.306
Infratentorial hemorrhage	14 (13.2%)	9 (16.1%)	5 (10.0%)	0.357
Subarachnoidal extension of hematoma	7 (6.6%)	5 (8.9%)	2 (4.0%)	0.308
Intraventricular hemorrhage	30 (28.3%)	20 (35.7%)	10 (20.0%)	0.073
Hemorrhage growth	20 (18.9%)	14 (25.0%)	6 (12.0%)	0.088
Early neurological deterioration	21 (19.8%)	16 (28.6%)	5 (10.0%)	0.017
GCS score	14 (13–15)	13 (12–14)	15 (14–15)	< 0.001
NIHSS score	10 (10−13)	13 (10–15)	8 (6–9)	< 0.001
ICH score	0 (0–1)	1 (0–2)	0 (0–1)	0.001
Hematoma volume (mL)	14 (7–21)	19 (14–26)	7 (4–12)	< 0.001
Systolic arterial pressure (mmHg)	170 ± 24	173 ± 23	166 ± 24	0.125
Diastolic arterial pressure (mmHg)	100 ± 10	101 ± 11	98 ± 10	0.091
Blood glucose level (mmol/L)	13.5 ± 4.3	14.4 ± 4.6	12.6 ± 3.7	0.033
Serum C-reactive protein level (mg/L)	13.3 ± 4.1	14.5 ± 4.2	12.0 ± 3.6	0.002
Blood white blood cell count ($\times 10^9$/L)	8.7 ± 2.8	9.1 ± 2.8	8.4 ± 2.8	0.203
Blood platelet count ($\times 10^9$/L)	179.2 ± 52.9	188.1 ± 62.3	169.2 ± 38.0	0.066
Serum NGAL levels > 417.6 ng/mL	62 (58.5%)	47 (83.9%)	15 (30.0%)	< 0.001

Continuous variables with normal distributions were presented as means with standard deviation, while those with skewed distributions were presented as medians with interquartile range. Categorical variables were presented as percentages. Statistical comparisons between different subgroups were performed using the chi-square test, Fisher exact test, unpaired t test, or Mann-Whitney U test as appropriate. NIHSS indicates National Institutes of Health Stroke Scale; ICH, intracerebral hemorrhage; mRS, modified Rankin Scale; NGAL, Neutrophil gelatinase-associated lipocalin; GCS, Glasgow coma scale.

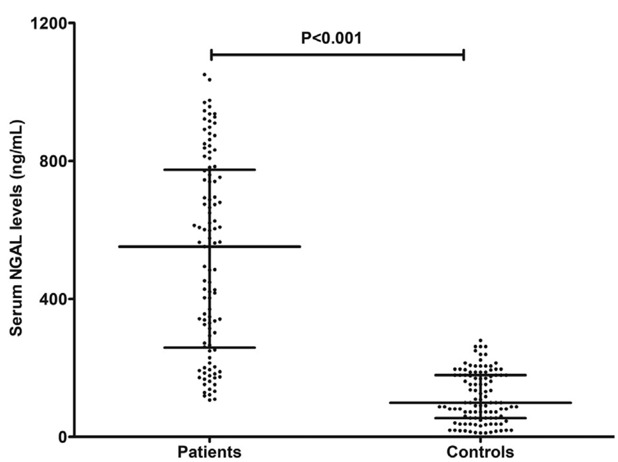

Fig. 1. Comparison of serum neutrophil gelatinase-associated lipocalin (NGAL) levels between controls and patients.

between serum NGAL levels and GCS scores, between serum NGAL levels and ICH scores, between serum NGAL levels and hematoma volumes, between serum NGAL levels and blood glucose levels, and also between serum NGAL levels and serum C-reactive proteins levels (Fig. 2). Alternatively, in Table 2, those patients who presented severe neurological dysfunction or severe ICH had substantially higher median serum NGAL levels than those who did not. Alternatively, the patients who had moderate-severe consciousness disturbance exhibited apparently higher median serum NGAL levels than those who did not. Finally, the median NGAL level was higher in patients with large hematoma volume as compared with the remainders.

3.3. Prognostic prediction

As just depicted in Fig. 3, serum NGAL levels were significantly higher in patients with unfavorable outcome than in those with favorable outcome. Under ROC curve, serum NGAL levels significantly discriminated the patients at risk of unfavorable outcome at 90 days after hemorrhagic stroke (AUC, 0.836; 95% CI, 0.751–0.901). In addition, an optimal cutoff value of serum NGAL levels for unfavorable outcome prediction was selected (417.6 ng/mL), which yielded the corresponding sensitivity and specificity values (Youden index J, 0.539; Fig. 4). Univariate and multivariate associations of clinical, biochemical and radiographic variables with 90-day unfavorable outcome are listed in Tables 1 and 3. The following variables were associated with unfavorable outcome on univariate analyses: age, admission body mass index, history of diabetes mellitus, presence of intraventricular hemorrhage, occurrence of hematoma growth, appearance of neurologic deterioration, baseline GCS score, initial NIHSS score, initial ICH score, admission hematoma volume, admission serum glucose, admission diastolic blood pressure, admission C-reactive protein levels, admission blood platelet count and admission serum NGAL levels. We incorporated the above-mentioned variables, which were found to be significant associations ($P < 0.1$), in the Logistic regression model, the multivariate regression analysis identified age, serum NGAL levels, NIHSS score and hematoma volume as the independent predictors for 90-day unfavorable outcome.

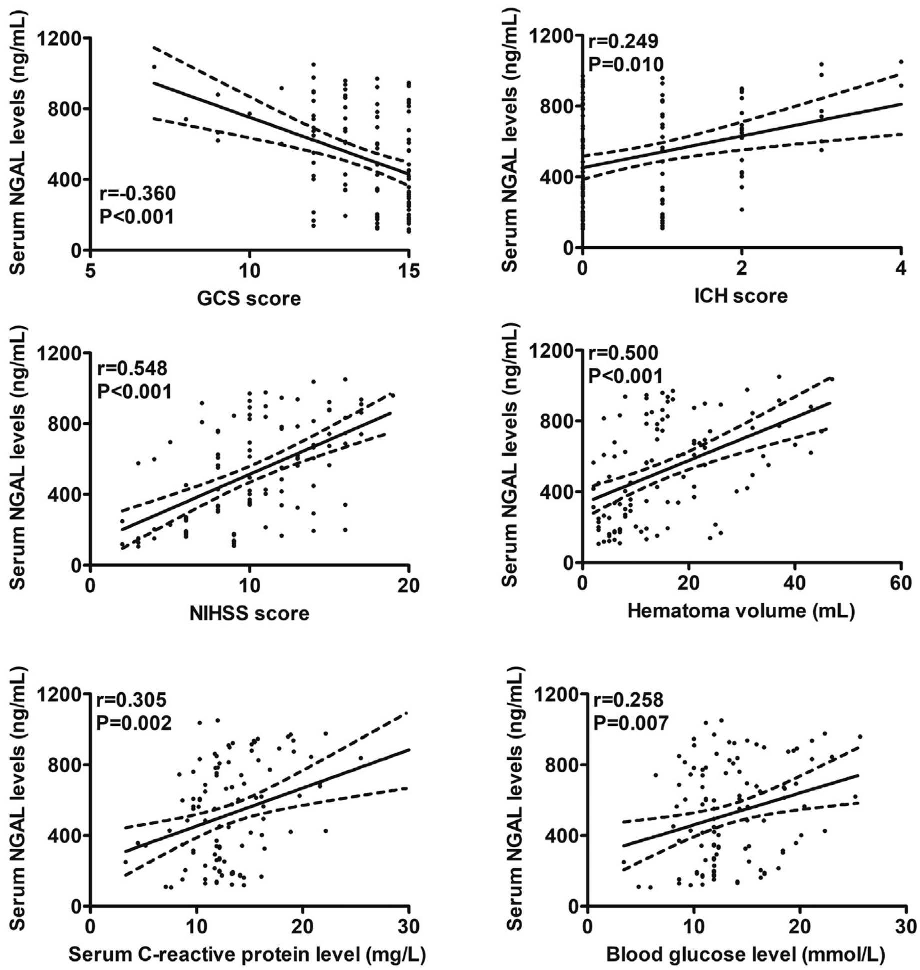

Fig. 2. Relationship between serum neutrophil gelatinase-associated lipocalin (NGAL) levels and Glasgow coma scale (GCS) score, intracerebral hemorrhage (ICH) score, National Institutes of Health Stroke Scale (NIHSS) score, hematoma volumes, serum C-reactive protein level and blood glucose level.

Table 2
Comparison of serum neutrophil gelatinase-associated lipocalin levels by subgroups in patients with acute spontaneous intracerebral hemorrhage.

Subgroups	Serum neutrophil gelatinase-associated lipocalin levels (ng/mL)	
	Median	Interquartile range
Severe neurological dysfunction (NIHSS score > 10)		
Presence	677.3	466.2 to 868.0
Absence	399.2	182.9 to 613.0
P value	< 0.001	
Severe intracerebral hemorrhage (ICH score > 2)		
Presence	844.0	670.6 to 1006.0
Absence	468.4	249.8 to 745.5
P value	0.003	
Moderate-severe consciousness disturbance (GCS score < 13)		
Presence	683.9	551.7 to 880.0
Absence	422.2	216.4 to 720.4
P value	0.004	
Large hematoma volume (Hematoma volume > 30 mL)		
Presence	760.0	610.6 to 898.0
Absence	428.5	221.9 to 718.2
P value	0.001	

Intergroup comparison was done using Mann-Whitney U test. NIHSS indicates National Institutes of Health Stroke Scale; ICH, intracerebral hemorrhage; GCS, Glasgow coma scale.

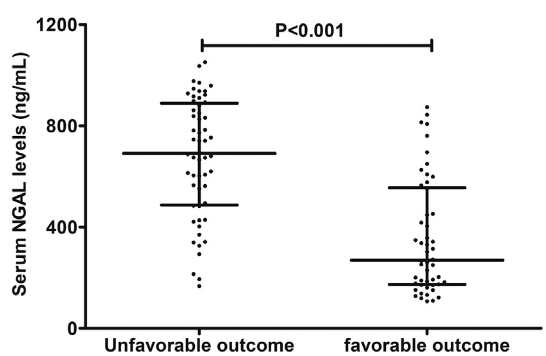

Fig. 3. Comparison of serum neutrophil gelatinase-associated lipocalin (NGAL) levels between intracerebral hemorrhage patients that were either at risk of unfavorable outcome or favorable outcome.

4. Discussion

To the best of my knowledge, this is the first study investigating serum NGAL levels in patients with ICH and subsequently revealing that (1) serum NGAL levels were substantially raised after hemorrhagic stroke, as compared with healthy controls; (2) serum NGAL levels were gradually elevated with increasing hemorrhagic severity; (3) serum NGAL emerged as an independent predictor for unfavorable outcome at 90 days after ICH; (4) under ROC curve, serum NGAL showed a significant predictive value for 90-day unfavorable outcome. Such data imply that serum NGAL might represent a promising prognostic

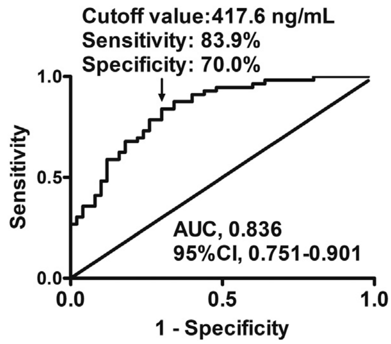

Fig. 4. Prognostic analysis of serum neutrophil gelatinase-associated lipocalin levels in patients with acute intracerebral hemorrhage in accordance with receiver operating characteristic curve.

biomarker for ICH.

ICH is one of the most common cerebrovascular diseases globally [1–3]. ICH may lead to persistent neurologic functional impairment or even death in a great number of patients [1–3]. Generally, mRS is clinically estimated to assess neurological functional outcome after ICH and commonly, mRS score > 2 is defined as an unfavorable outcome [4,6,9,19]. Moreover, follow-up time of 3 months or 90 days is appreciated [4,6,9,19]. In the current study, the ICH patients were followed up until death or complete of 90 days after hemorrhagic stroke. The percentage of unfavorable outcome varies according to the difference of age, gender, race, treatment or follow-up time, which is almost 50% [4,6,9,19]. In this study, we reported the similar result for incidence of unfavorable outcome, which was 52.8%. Taken together, ICH can actually result in severe disability among survivors after stroke.

Stroke patients are especially prone to inflammatory processes. Multiple pathophysiological pathways have been made responsible for inflammatory response underlying hemorrhagic brain injury. Pro-inflammatory cytokines such as interleukins −1, −6, and tumor necrosis factor -α as well as chemokines and adhesion molecules have been shown to contribute to brain injury, thereby causing neurologic impairment [36–38]. NGAL, a siderophore-binding protein, is involved in cellular iron transport [20–24]. Of interest, it also acts as an acute phase protein that is up-regulated in a variety of central nervous system injuries. The accumulating evidence has shown that NGAL plays an essential role in brain edema formation, blood-brain barrier disruption, neurologic deficits, neuroinflammation and neuronal death in rats after thrombin-induced brain injury, intraventricular haemoglobin-induced brain injury and intracerebral injection of autologous blood –induced brain injury [25–28]. The current study found a substantial elevation of serum NGAL levels after hemorrhagic stroke; of note, it was revealed that serum NGAL levels were positively correlated with serum C-reactive protein levels and blood glucose levels, which are the two biomarkers systemically reflecting acute reactive status after ICH [12,39,40]. In conclusion, serum NGAL might emerge as an acute phase protein, which was presumed to be related to inflammation, for assessment of adverse events following hemorrhagic stroke.

Recently, it was reported that NGAL mRNA and protein levels were profoundly raised in human traumatized brain tissue; also serum NGAL levels were pronouncedly enhanced in patients with traumatic brain injury; intriguingly, NGAL levels had an inverse and significant correlation with GCS score [29]. In view of the preceding results, authors suggested that NGAL might be a novel biomarker reflecting head trauma severity [29]. It was our speculation that NGAL levels might be correlated with hemorrhagic severity after ICH. In the current study, hemorrhagic severe were assessed using four clinical, radiological or comprehensive parameters, i.e., NIHSS score, ICH score, hematoma volume and GCS score; meanwhile, they were dichotomized based on

Table 3
Univariable and multivariable logistic regression analyses evaluating the association of baseline characteristics with the likelihood of 90-day unfavorable outcome (mRS > 2) in patients with acute spontaneous intracerebral hemorrhage.

Factors	Univariable analysis		Multivariable analysis	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Gender (Male/Female)	1.036 (0.479, 2.241)	0.929		
Age (y)	1.044 (1.002, 1.087)	0.040	1.116 (1.012, 1.231)	0.028
Body mass index (kg/m^2)	1.160 (0.979, 1.376)	0.087	1.109 (0.903, 1.362)	0.322
Current smoking	1.009 (0.470, 2.164)	0.982		
Moderate-heavy alcohol consumption	1.565 (0.712, 3.440)	0.265		
Hypertension	0.818 (0.263, 2.545)	0.729		
Diabetes mellitus	2.588 (1.078, 6.216)	0.033	2.537 (0.909, 7.080)	0.075
Hyperlipidemia	0.906 (0.411, 1.998)	0.808		
Congestive heart failure	2.880 (0.554, 14.975)	0.209		
Coronary artery disease	1.643 (0.451, 5.984)	0.452		
Chronic kidney disease	1.880 (0.445, 7.951)	0.391		
Statin pretreatment	1.380 (0.582, 3.273)	0.464		
Lobar hemorrhage	1.600 (0.648, 3.949)	0.308		
Infratentorial hemorrhage	1.723 (0.536, 5.537)	0.361		
Subarachnoidal extension of hematoma	2.353 (0.436, 12.708)	0.320		
Intraventricular hemorrhage	2.222 (0.919, 5.371)	0.076	3.636 (0.819, 16.150)	0.090
Hemorrhage growth	2.444 (0.859, 6.955)	0.094	1.447 (0.418, 5.005)	0.560
Early neurological deterioration	3.600 (1.209, 10.716)	0.021	3.110 (0.880, 10.999)	0.078
GCS score	0.452 (0.310, 0.660)	< 0.001	0.506 (0.186, 1.379)	0.183
NIHSS score	1.822 (1.457, 2.277)	< 0.001	1.853 (1.385, 2.479)	< 0.001
ICH score	2.157 (1.343, 3.464)	0.001	1.807 (0.712, 4.585)	0.213
Hematoma volume (mL)	1.165 (1.091, 1.243)	< 0.001	1.281 (1.107, 1.483)	0.001
Systolic arterial pressure (mmHg)	1.013 (0.996, 1.030)	0.127		
Diastolic arterial pressure (mmHg)	1.033 (0.994, 1.074)	0.094	0.960 (0.881, 1.046)	0.351
Blood glucose level (mmol/L)	1.109 (1.006, 1.223)	0.037	1.056 (0.935, 1.193)	0.377
Serum C-reactive protein level (mg/L)	1.195 (1.060, 1.347)	0.004	1.098 (0.936, 1.288)	0.253
Blood white blood cell count ($\times 10^9$/L)	1.096 (0.952, 1.261)	0.202		
Blood platelet count ($\times 10^9$/L)	1.007 (0.999, 1.015)	0.070	0.988 (0.975, 1.002)	0.082
Serum NGAL levels > 417.6 ng/mL	12.185 (4.783, 31.041)	< 0.001	4.368 (1.160, 16.455)	0.029

NIHSS indicates National Institutes of Health Stroke Scale; ICH, intracerebral hemorrhage; mRS, modified Rankin Scale; OR, odds ratio; 95% CI, 95 confidence interval; NGAL, Neutrophil gelatinase-associated lipocalin; GCS, Glasgow coma scale.

the previous definition, consequently which emerged as categorical variables. Notably, we found an intimate relationship between serum NGAL levels and those severity parameters, which were identified as either the continuous or categorical variables. Overall, serum NGAL might serve as a potential biomarker for reflecting hemorrhagic severity after ICH.

In order to investigate the relationship between serum NGAL levels and prognosis at 90 days after hemorrhagic stroke, we attempted to include variables known to influence prognosis in univariate analysis. A ROC curve was established and a suitable cutoff value was set at 417.6 ng/mL, which predicted 90-day unfavorable outcome with a sensitivity at 83.9% and a specificity at 70.0%. Afterwards, serum NGAL level was dichotomized in accordance with 417.6 ng/mL. We contained all variables, which were verified to be significantly associated with 90-day unfavorable outcome in univariate analyses, in a multivariate model and thereby it was confirmed that, besides age, NIHSS score and hematoma volume, serum NGAL levels > 417.6 ng/mL emerged as an independent predictor for such a poor prognosis. This model had included so many confounding variables listed in Tables 1 and 3. And, there is a relatively large sample size of 106 patients. Hence, it was assumed that serum NGAL should represent a promising prognostic biomarker of hemorrhagic stroke.

5. Conclusions

In summary, NGAL levels are elevated in serum from patients with ICH and its serum levels are closely correlated to GCS and NIHSS scores, as well as ICH score and hematoma volume; simultaneously, serum NGAL is identified as an independent predictor for 90-day unfavorable outcome following hemorrhagic stroke. Given that serum NGAL levels are in profound association with the severity and poor prognosis of ICH, serum NGAL might be of important prognostic value.

Acknowledgements

The authors thank all staffs in Department of Neurosurgery, Department of Emergency Medicine, Department of Emergency Intensive Care Unit and Department of Neurology, Ruian People's Hospital (Ruian, China) for their technical support.

Competing interests

The authors declare that they have no competing interests.

References

[1] M.I. Aguilar, W.D. Freeman, Spontaneous intracerebral hemorrhage, Semin. Neurol. 30 (2010) 555–564.
[2] S. Chan, J.C. Hemphill 3rd., Critical care management of intracerebral hemorrhage, Crit. Care Clin. 30 (2014) 699–717.
[3] J.J. Provencio, I.R. Da Silva, E.M. Manno, Intracerebral hemorrhage: new challenges and steps forward, Neurosurg. Clin. N. Am. 24 (2013) 349–359.
[4] G.R. Zheng, B. Chen, J. Shen, S.Z. Qiu, H.M. Yin, W. Mao, et al., Serum myeloperoxidase concentrations for outcome prediction in acute intracerebral hemorrhage, Clin. Chim. Acta 487 (2018) 330–336.
[5] Y.Y. Hu, X.Q. Dong, W.H. Yu, Z.Y. Zhang, Change in plasma S100B level after acute spontaneous basal ganglia hemorrhage, Shock 33 (2010) 134–140.
[6] B. Chen, J. Shen, G.R. Zheng, S.Z. Qiu, H.M. Yin, W. Mao, et al., Serum cyclophilin a concentrations and prognosis of acute intracerebral hemorrhage, Clin. Chim. Acta 486 (2018) 162–167.
[7] J. Satopää, S. Mustanoja, A. Meretoja, J. Putaala, M. Kaste, M. Niemelä, et al., Comparison of all 19 published prognostic scores for intracerebral hemorrhage, J. Neurol. Sci. 379 (2017) 103–108.
[8] C. Finocchi, M. Balestrino, L. Malfatto, G. Mancardi, C. Serrati, C. Gandolfo, National institutes of health stroke scale in patients with primary intracerebral hemorrhage, Neurol. Sci. (2018), https://doi.org/10.1007/s10072-018-3495-y.
[9] S.Q. Qian, S.R. He, B.B. Li, J. Qian, X.D. Zheng, Serum S100A12 and 30-day mortality after acute intracerebral hemorrhage, Clin. Chim. Acta 477 (2018) 1–6.
[10] J.E. Bernstein, P. Savla, F. Dong, B. Zampella, J.G. Wiginton 4th, D.E. Miulli, et al., Inflammatory markers and severity of intracerebral hemorrhage, Cureus 10 (2018) e3529.
[11] L.R. Øie, M.A. Madsbu, O. Solheim, A.S. Jakola, C. Giannadakis, A. Vorhaug, et al., Functional outcome and survival following spontaneous intracerebral hemorrhage: a retrospective population-based study, Brain Behav. 8 (2018) e01113.
[12] J. Rosenthal, A. Lord, K. Ishida, J. Torres, B.M. Czeisler, A. Lewis, Highest in-hospital glucose measurements are associated with neurological outcomes after intracerebral hemorrhage, J. Stroke Cerebrovasc. Dis. 27 (2018) 2662–2668.
[13] J.C. Hemphill 3rd, D.C. Bonovich, L. Besmertis, G.T. Manley, S.C. Johnston, The ICH score: a simple, reliable grading scale for intracerebral hemorrhage, Stroke 32 (2001) 891–897.
[14] T. Nisar, A. Alchaki, M. Hillen, Validation of ICH score in a large urban population, Clin. Neurol. Neurosurg. 174 (2018) 36–39.
[15] J.C. Hemphill 3rd, M. Farrant, T.A. Neill Jr., Prospective validation of the ICH score for 12-month functional outcome, Neurology 73 (2009) 1088–1094.
[16] X. Lan, X. Han, X. Liu, J. Wang, Inflammatory responses after intracerebral hemorrhage: from cellular function to therapeutic targets, J. Cereb. Blood Flow Metab. (2018), https://doi.org/10.1177/0271678X18805675.
[17] Y. Cheng, J. Zan, Y. Song, G. Yang, H. Shang, W. Zhao, Evaluation of intestinal injury, inflammatory response and oxidative stress following intracerebral hemorrhage in mice, Int. J. Mol. Med. 42 (2018) 2120–2128.
[18] P. Li, C. Jiwu, Butin attenuates brain edema in a rat model of intracerebral hemorrhage by anti inflammatory pathway, Transl. Neurosci. 9 (2018) 7–12.
[19] Q. Lin, J.Y. Cai, C. Lu, J. Sun, H.J. Ba, M.H. Chen, et al., Macrophage migration inhibitory factor levels in serum from patients with acute intracerebral hemorrhage: potential contribution to prognosis, Clin. Chim. Acta 472 (2017) 58–63.
[20] L. Kjeldsen, D.F. Bainton, H. Sengeløv, N. Borregaard, Identification of neutrophil gelatinase-associated lipocalin as a novel matrix protein of specific granules in human neutrophils, Blood 83 (1994) 799–807.
[21] J.B. Cowland, N. Borregaard, Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans, Genomics 45 (1997) 17–23.
[22] R.K. Strong, T. Bratt, J.B. Cowland, N. Borregaard, F.C. Wiberg, A.J. Ewald, Expression, purification, crystallization and crystallographic characterization of dimeric and monomeric human neutrophil gelatinase associated lipocalin (NGAL), Acta Crystallogr. D Biol. Crystallogr. 54 (1998) 93–95.
[23] T.H. Flo, K.D. Smith, S. Sato, D.J. Rodriguez, M.A. Holmes, R.K. Strong, et al., Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron, Nature 432 (2004) 917–921.
[24] L.R. Devireddy, C. Gazin, X. Zhu, M.R. Green, A cell-surface receptor for lipocalin 24p3 selectively mediates apoptosis and iron uptake, Cell 123 (2005) 1293–1305.
[25] W. Ni, M. Zheng, G. Xi, R.F. Keep, Y. Hua, Role of lipocalin-2 in brain injury after intracerebral hemorrhage, J. Cereb. Blood Flow Metab. 35 (2015) 1454–1461.
[26] M. Dong, G. Xi, R.F. Keep, Y. Hua, Role of iron in brain lipocalin 2 upregulation after intracerebral hemorrhage in rats, Brain Res. 1505 (2013) 86–92.
[27] H. Shishido, Y. Toyota, Y. Hua, R.F. Keep, G. Xi, Role of lipocalin 2 in intraventricular haemoglobin-induced brain injury, Stroke Vasc. Neurol. 1 (2016) 37–43.
[28] S. Mao, G. Xi, R.F. Keep, Y. Hua, Role of lipocalin-2 in thrombin-induced brain injury, Stroke 47 (2016) 1078–1084.
[29] J. Zhao, H. Chen, M. Zhang, Y. Zhang, C. Qian, Y. Liu, et al., Early expression of serum neutrophil gelatinase-associated lipocalin (NGAL) is associated with neurological severity immediately after traumatic brain injury, J. Neurol. Sci. 368 (2016) 392–398.
[30] R.U. Kothari, T. Brott, J.P. Broderick, W.G. Barsan, L.R. Sauerbeck, M. Zuccarello, et al., Et al. the ABCs of measuring intracerebral hemorrhage volumes, Stroke 27 (1996) 1304–1305.
[31] T. Brott, J. Broderick, R. Kothari, W. Barsan, T. Tomsick, L. Sauerbeck, et al., Early hemorrhage growth in patients with intracerebral hemorrhage, Stroke 28 (1997) 1–5.
[32] N. Goyal, G. Tsivgoulis, K. Malhotra, A.L. Houck, Y.M. Khorchid, A. Pandhi, et al., Serum magnesium levels and outcomes in patients with acute spontaneous intracerebral hemorrhage, J. Am. Heart Assoc. 7 (2018) (pii: e008698).
[33] G. Tsivgoulis, V.A. Lioutas, P. Varelas, A.H. Katsanos, N. Goyal, R. Mikulik, et al., Direct oral anticoagulant- vs vitamin K antagonist-related nontraumatic intracerebral hemorrhage, Neurology 89 (2017) 1142–1151.
[34] S.J. Gu, H.F. Xuan, M. Lu, X.Z. Chen, W.F. Dong, X.F. Yan, et al., Admission plasma visfatin level strongly correlates with hematoma growth and early neurologic deterioration in patients with acute spontaneous basal ganglia hemorrhage, Clin. Chim. Acta 425 (2013) 85–89.
[35] J.L. Banks, C.A. Marotta, Outcomes validity and reliability of the modified Rankin scale: implications for stroke clinical trials: a literature review and synthesis, Stroke 38 (2007) 1091–1096.
[36] M.H. Askenase, L.H. Sansing, Stages of the inflammatory response in pathology and tissue repair after intracerebral hemorrhage, Semin. Neurol. 36 (2016) 288–297.
[37] H. Zheng, C. Chen, J. Zhang, Z. Hu, Mechanism and therapy of brain edema after intracerebral hemorrhage, Cerebrovasc. Dis. 42 (2016) 155–169.
[38] S. Chen, Q. Yang, G. Chen, J.H. Zhang, An update on inflammation in the acute phase of intracerebral hemorrhage, Transl. Stroke Res. 6 (2015) 4–8.
[39] P. Löppönen, C. Qian, S. Tetri, S. Juvela, J. Huhtakangas, M.K. Bode, et al., Predictive value of C-reactive protein for the outcome after primary intracerebral hemorrhage, J. Neurosurg. 121 (2014) 1374–1379.
[40] M. Di Napoli, D.A. Godoy, V. Campi, L. Masotti, C.J. Smith, A.R. Parry Jones, et al., C-reactive protein in intracerebral hemorrhage: time course, tissue localization, and prognosis, Neurology 79 (2012) 690–699.