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Anion gap predicts the long-term neurological and cognitive outcomes of spontaneous intracerebral hemorrhage

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Abstract. – OBJECTIVE: To investigate whether anion gap (AG) can act as a potentially predictive biomarker in recoveries of neurological and cognitive functions.

PATIENTS AND METHODS: A total of 89 patients with intracerebral hemorrhage (ICH) were recruited. Of these, 68 and 21 patients were categorized into screening cohort and validation cohort, respectively. In the screening cohort, patients were categorized into three groups, according to the serum AG levels at admission. We dynamically recorded AG levels. Neurological and cognitive functions were assessed using Glasgow coma scale (GCS), Glasgow outcome scale (GOS) and mini-mental state examination (MMSE) scale at different time points. Furthermore, in the validation cohort, 9 patients with increased AG level underwent interventions to rectify the electrolyte imbalance.

RESULTS: In the screening cohort, statistical differences were observed for respiratory diseases (p=0.029) among the three groups. The number of patients in the ≥16 mmol/L group (59.3%) was higher than that in the other groups. The mean scores of GCS in the ≥16 mmol/L group were lower than those in the other groups. The AG levels at admission had significant associations with 180-day GOS (p=0.043) and 180-day MMSE (p=0.001). Among them, the mean scores of the 180-day GOS and 180-day MMSE were lower in the ≥16 mmol/L group than in the other groups. In the validation cohort, AG intervention promoted recoveries of neurological and cognitive functions when compared to those without AG interventions.

CONCLUSIONS: AG is a potentially predictive biomarker for the long-term outcomes of ICH patients, and rectifying AG at admission improves the outcomes.

Key Words:
Intracerebral hemorrhage, Anion gap, Prognosis, Acid-base balance, Electrolyte.

Introduction

Spontaneous intracerebral hemorrhage (sICH) is a severe cerebrovascular event, with a one-month mortality of about 40% in the acute phase of the disease. Although sICH only accounts for about 10-15% in all stroke patients, the high incidences of mortality and disability indicate an urgent need for the investigation of preventive and therapeutic strategies[1]. In recent years, the age of onset of ICH has tended to be young. However, many patients have a history of underlying diseases such as hypertension, diabetes, hyperlipidemia, and respiratory diseases. These diseases influence the pathogenesis of ICH and impact the recovery status[2]. Therefore, interventions for such underlying diseases and stabilization of homeostasis play extremely important roles in ICH.

The clinical diagnosis of sICH mainly depends on the neuroimage evidence, such as computed tomography (CT) and magnetic resonance image (MRI). These tests provide a rapid recognition of sICH and differential diagnosis from other cerebrovascular diseases, such as ischemic stroke (IS) and subarachnoid hemorrhage (SAH), with high specificity and sensitivity[3]. However, the neuroimages can neither distinguish the microenvironments of body fluid nor have predictive values for the neurological complications of the patients. Therefore, numerous studies have focused on exploring novel biomarkers for predicting the outcomes in ICH[4,5]. Several serum or plasma molecules have been considered to be significantly correlated with the neurological and/or cognitive impairments after ICH onset. However, most of these molecule-based studies have only been proof-of-concept so far, and have not been introduced in routine clinical practice. Hence, it is imperative to develop a clinical routine-based model to predict patient outcome in ICH.

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Anion gap (AG) is an algorithmic parameter that calculates the difference between serum cation and anion concentrations, and is based on the following equation: AG=[Na^+(mmol/L)+K^+(mmol/L)]−[Cl^-(mmol/L)+HCO_3^-(mmol/L)]. AG reflects the acid-base equilibrium in body fluids and plays a pivotal role in metabolic acidosis and ischemic anoxic encephalopathy[6]. In recent studies[7], serum AG has been investigated to be associated with survival rate after the acute phase of ischemic stroke in a rodent model. In another clinical study[8], a high level of AG was significantly correlated to an increased risk of all-cause mortality and short-term prognosis in cerebral infarction patients.

Although AG has been investigated in some stroke subtypes, we found little information about the predictive values of AG in ICH. Hence, we performed a clinical study to investigate the correlation of AG levels at admission with the neurological and cognitive impairments in both short- and long-term outcomes of ICH. Based on the retrospective evidence, we performed an interventional study for AG levels in a small cohort to determine whether rectifying AG levels could promote the recoveries.

Patients and Methods

Study Population

We performed a retrospective hospital-based case-control study. A total of 89 patients were recruited from Lu'an Second People's Hospital. All patients underwent CT and/or MRI examinations at admission and were diagnosed with ICH by two senior neurosurgeons. Of these, 68 and 21 patients were categorized into the screening cohort and validation cohort, respectively. Patients who had cancers, autoimmune diseases, systemic diseases, or other intracranial diseases were excluded from the study. The 68 patients in the screening cohort were divided into three groups, according to the AG levels. In the validation cohort, the AG levels for all patients were above 16 mmol/L. 9 among those patients received interventions to rectify the AG level to be <16 mmol/L. For the remaining 12 patients, AG levels were >16 mmol/L, but all ions contributing to the AG level were just abnormal in a mild-to-moderate manner.

Clinical Characteristics and Sample Collection

Demographic data and clinical characteristics were collected for all patients, including age, sex, history of diseases and medications, ICH volume, midline shift, and intraventricular hemorrhage (IVH). Meanwhile, all patients were examined for neurological damage, assessed by Glasgow coma scale (GCS) at admission. Recovery outcomes were assessed by Glasgow outcome scale (GOS) and mini-mental state examination (MMSE) scale at the 1st and 3rd month, respectively. Scoring evaluations were conducted by two experienced senior doctors. Sera were drawn by forearm venipuncture into evacuated EDTA-coated tubes from each patient at different time points at day 0 (the day of admission and within 4 hours from the disease onset), day 1, day 3, day 7, and day 14 after admission. All serum samples were stored at -80°C until further analysis.

Treatments

All patients recruited for the study received neurosurgical treatments for hematoma removal. Other adjuvant therapeutic treatments included drainage of CSF by lumbar puncture or lumbar cistern to reduce intracranial pressure, application of calcium channel blocker, application of mannitol to relieve cerebral edema and application of neurotrophic drugs. In the validation cohort, electrolyte rectification was carried out for the intervention group at admission.

Statistical Analysis

We used SPSS (version 19, IBM Corp., Armonk, NY, USA) software for statistical analysis. Binary data were analyzed using chi-squared test. The enumeration data were analyzed by using Kruskal-Wallis test or Student's *t*-test between two groups and by the one-way ANOVA analysis when the numbers of group >3. The *p*-values reported in the study were based on a two-sided probability test with a significance level of $p<0.05$.

Results

Demographic Features and Grouping by AG levels

In the screening cohort, the patients were divided three groups according to the AG levels: AG<12 mmol/L group, 12 mmol/L≤AG<16 mmol/L (12-16 mmol/L group) group, and AG≥16 mmol/L group. The demographic features and clinicopathological data for the patients in the three groups are summarized in Table I. The numbers of respiratory diseases were significantly different among the three groups ($p=0.029$). Among them, the pro-

Table I. Demographic and clinicopathological information for intracerebral hemorrhage patients.

		Screening group (n=68)				Validation group (n=21)		
		AG<12 mmol/L (n=16)	AG: 12-16 mmol/L (n=25)	AG≥16 mmol/L (n=27)	*p*-value	Intervention (n=9)	Non-intervention (n=12)	*p*-value
Age		65.19±12.69	65.72±14.93	65.07±11.71	0.983	63.56±5.77	66.00±7.63	0.433
Gender					0.754			0.660
	Male	9 (56.3%)	12 (48.0%)	12 (44.4%)		3 (33.3%)	6 (50.0%)	
	Female	7 (43.7%)	13 (52.0%)	15 (55.6%)		6 (66.7)	6 (50.0%)	
Underlying diseases								
	Hypertension	13 (81.3%)	20 (80.0%)	19 (70.4%)	0.627	8 (88.9%)	8 (66.7%)	0.338
	T2DM	5 (31.3%)	12 (48.0%)	7 (25.9%)	0.232	4 (44.4%)	7 (58.3%)	0.670
	Hyperlipidemia	7 (43.8%)	10 (40.0%)	15 (55.6%)	0.508	3 (33.3%)	3 (25.0%)	1.000
	Respiratory disease	4 (25.0%)	9 (36.0%)	16 (59.3%)	**0.029**	6 (66.7%)	5 (41.7%)	0.387
Hospital stay (day)		15.25±3.53	16.76±4.00	17.11±3.61	0.278	18.11±4.34	17.25±4.20	0.652
GCS		10.13±2.24	10.44±2.45	9.19±2.18	0.135	10.44±1.94	9.67±1.72	0.344
Bleeding volume (cm^3)		22.50±7.24	24.56±6.51	24.26±6.65	0.608	24.44±7.50	24.17±6.10	0.926
Midline shift, >10 mm		3 (18.8%)	7 (28.0%)	9 (33.3%)	0.588	3 (33.3%)	5 (41.7%)	1.000
IVH		5 (31.3%)	8 (32.0%)	9 (33.3%)	0.989	4 (44.4%)	5 (41.7%)	1.000
Serum ion levels at admission (mmol/L)								
	Na^+	138.28±7.04	140.24±5.33	139.75±6.06	0.555	140.93±4.62	141.45±4.48	0.799
	K^+	3.99±1.16	4.39±1.06	4.61±1.26	0.372	4.14±0.96	4.32±1.09	0.702
	Cl^-	103.02±6.85	105.13±5.81	101.40±5.87	0.078	101.82±7.28	104.80±5.40	0.294
	HCO_3^-	28.06±3.53	25.34±3.82	25.97±4.07	0.058	26.44±4.22	24.33±3.61	0.232
Serum AG level at admission (mmol/L)		11.20±0.56	14.15±0.86	16.98±0.65	**<0.001**	16.81±0.40	16.64±0.39	0.332

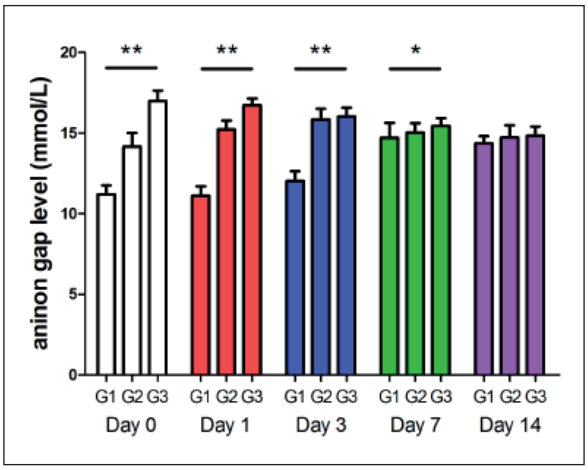

Figure 1. Continuous alterations of anion gap levels of patients in the screening cohort. (G1: AG<12 mmol/L; G2: AG=12-16 mmol/L; G3: AG>16 mmol/L).

portion of patients with respiratory diseases was higher in the AG>16 mmol/L group than in the other two groups. We found no statistical differences with respect to age, sex, underlying hypertension, type 2 diabetes mellitus (T2DM), hyperlipidemia, hospital stay, bleeding volume, midline shift, and GCS score at admission in the screening cohort. In another aspect, there was no statistical difference in the clinical parameters between the intervention and non-intervention groups in the validation cohort.

Dynamic Changes of AG Level After Neurosurgery

We tested the dynamic changes of serum AG levels among the patients in the screening cohort at admission (day 0) and day 1-14 post-surgery (days 1, 3, 7, and 14). If the patients were discharged from the hospital less than 14 days (but more than 10 days) after surgery, the last time point for sample collection was considered to be the sampling time at day 14. As shown in Figure 1, there were significant differences of serum AG levels among the three groups at admission (p<0.001), day 1 (p<0.001), day 3 (p<0.001), and day 7 (p=0.002), but the serum AG levels reached normal levels on day 14 with no statistical difference (p=0.067).

AG Level at Admission Predicts Recovery Status of ICH Patients

We assessed the recoveries of neurological and cognitive functions among all ICH patients at day 30 and 180 using the GOS (30-day and 180-day GOS) and MMSE (30-day and 180-day MMSE) scales. Comparing the recovery status of the 3 groups, we found that the serum AG levels at admission were significantly correlated with 180-day GOS (p=0.043) and 180-day MMSE (p=0.001). The mean scores of 180-day GOS and 180-day MMSE at AG>16 mmol/L group were significantly lower than those in the other two groups (Table II).

Rectifying Electrolytes at Admission Facilitates to Promote Recovery

In the validation cohort, 21 ICH patients had abnormal AG levels. An intervention for rectifying the AG level was performed in nine patients. Then, all the patients in the validation cohort received neurosurgery for the treatment of ICH. We assessed the GOS and MMSE scales at day 180 and found significant differences between the intervention and non-intervention group for the 180-day GOS (p=0.011) and 180-day MMSE (p=0.002), indicating that rectifying serum AG level at admission might promote the long-term outcomes in ICH patients (Table III).

Discussion

In this study, we retrospectively analyzed the relationship between the AG levels at admission with short- and long-term outcomes of patients with ICH. The results indicated that the proportion of patients who had respiratory diseases was higher in the AG>16 mmol/L group compared to those in the other two groups in which AG levels were lower than 16 mmol/L. Furthermore, we also

Table II. Relationship between AG and patient outcomes at 180-day following up in the screening cohort.

Scales	AG (mmol/L)			*p*-value
	<12 (n=16)	12-16 (n=25)	≥16 (n=27)	
30-day GOS	3.31±0.79	3.28±0.74	3.33±0.02	0.973
180-day GOS	4.37±0.72	3.96±0.79	3.74±0.81	**0.043**
30-day MMSE	16.81±4.94	15.48±3.50	15.06±4.34	0.053
180-day MMSE	24.56±3.98	20.16±4.04	19.74±3.86	**0.001**

Table III. Neurological and recognition outcomes in the validation cohort.

Scales	Intervention group	Non-intervention group	*p*-value
180-day GOS	4.33±0.50	3.58±0.67	**0.011**
180-day MMSE	21.11±3.14	16.67±2.64	**0.002**

found that the group with AG>16 mmol/L had lower scores for 180-day GOS and 180-day MMSE than those in the other two groups. These results indicated that the higher level of AG at admission predicted long-term poor outcomes in neurological and cognitive functions after ICH.

Because ICH usually leads to high rates of mortality and disability, accurate and timely diagnosis is the most important issue after the occurrence. Neuroimaging indications, which are provided by CT and MRI scans, have been widely applied clinically and could provide rapid diagnosis of ICH. However, neuroimaging technology also has some limitations in clinical practice. First, only 50% of patients with progressive bleeding would be positively detected by imageological findings[9,10]. Second, these technologies hardly offer a predictive value for the prognosis of patients. Therefore, researchers currently focus on the molecule-based study in stroke, which provides important clinical clues for not only precise diagnosis and outcome prediction of the disease, but also pathogenesis investigations on ICH.

Elevated serum AG level results from the production of increased organic acid or decreased anions. Serum lactate and pyruvate are considered to be causative for the pathological elevation of AG level[11]. A previous study demonstrated that the level of serum lactate was significantly correlated to patients' outcomes in the intensive care unit (ICU), especially in terms of long-term mortality[12]. However, serum lactate is often influenced by numerous factors such as temperature, sample storage time, and infections[13]. Therefore, other potential alternatives for lactate in prediction of diseases status and outcomes should be considered. In some clinical studies, the serum AG level is reported to have negative correlations with some diseases. Chen et al[14] performed a multivariate analysis and found that the increased serum AG level at admission was correlated to the mortality of the aortic aneurysm in the ICU. Yang et al[15] found that higher AG levels indicated a severity of coronary heart diseases and was positively correlated to the deterioration of cardiovascular functions. Furthermore, Liu et al[8] performed a 1-year follow-up study for 1,113 patients with IS who were admitted to the ICU and found that serum AG levels were significantly correlated to increased risks of all-cause mortality and short-term prognosis in patients with cerebral infarctions.

In our study, we found the proportion of patients with AG>16 mmol/L had lower neurological and cognitive scores compared to those in the other two groups, in which the serum AG levels were <16 mmol/L. Meanwhile, the patients in the AG>16 mmol/L group had a higher proportion of respiratory diseases. We inferred that these patients might have underlain metabolic acidosis before or at the onset of ICH. Morgan et al[16] reviewed 30 relative studies concerning the relationship between chronic obstructive pulmonary diseases (COPD) and cerebrovascular diseases and found that the prevalence and incidence of stroke increased in people with COPD. Meanwhile, de Miguel-Diez et al[17] found that COPD was associated with an increased risk of in-hospital mortality among IS patients, indicating that respiratory diseases might deteriorate overall recovery. However, whether rectification of acidosis could alleviate the neurological and cognitive impairments has still not been fully elaborated. We therefore prospectively performed interventions for patients who were diagnosed with respiratory diseases to rectify the acidosis at admission or before surgery. The follow-up results showed that rectification of acidosis could alleviate the neurological and cognitive impairments for the long-term outcomes of ICH patients.

In previous studies, serum electrolytes or ions played important roles in the course of ICH. Sallinen et al[18] found that hypocalcemia was associated with larger hematoma volumes among ICH patients and therefore induced a higher mortality rate in comparison with the normocalcemic patients. Rajendran et al[19] found that the level of serum ferritin increased in ICH and was significantly correlated to the outcomes. Compared to single electrolytes or ions, AG reflects a comprehensive level of multiple ions, representing an acid-base equilibrium status in the body. Therefore, it could have a comprehensive assessment of diseases.

Although there was still lack of valid evidence for the correlation between ICH onset and the underlying metabolic acidosis, we found that maintaining the acid-base balance at the early stage of

ICH could avoid an alleviation of secondary injury and thus probably promoted the recoveries of neurological and cognitive functions.

This study has some limitations. First, the number of participants in this study was relatively small, especially of those in the validation cohort. Because ICH patients typically undergo emergency operation, the perioperative management is often carried out urgently, when a mild-to-moderate electrolyte disturbance seems acceptable. Therefore, the numbers of patient with efficacious interventions might be limited. Second, in the follow-up study, we only assessed the neurological and cognitive functions for patients but did not screen the follow-up AG levels. Chronic abnormality of AG level in the recovery stage would be another important issue which should be carefully noticed. We are now warranted to perform the study on the topic.

Conclusions

In summary, screening of serum AG levels at admission in ICH patients provides an assessment of acid-base equilibrium of body fluids and predicts the neurological and cognitive functions in the long-term outcomes of ICH patients. Interventions for maintaining the acid-base balance in the early stage of ICH can promote the long-term recovery of ICH patients.

Informed Consent

All subjects provided informed consents.

Ethical Approval

This study was approved by the Clinical Research Ethics Committee of the hospital and conducted according to the tenets of the Declaration of Helsinki.

Conflict of Interests

The authors declare that they have no conflict of interests.

References

1) Krishnamurthi RV, Moran AE, Forouzanfar MH, Bennett DA, Mensah GA, Lawes CMM, Barker-Collo S, Connor M, Roth GA, Sacco R, Ezzati M, Naghavi M, Murray CJL, Feigin VL, Global Burden of DIseases, Injuries, And Risk Factors 2010 Study Stroke Expert Group. The global burden of hemorrhagic stroke: a summary of findings from the GBD 2010 study. Glob Heart 2014; 9: 101-106.
2) Mota LAT, Silva DRB, Pfeifer LI. Functional outcomes in children related to self-care, mobility, and social function after stroke in early childhood: a cohort study. Arq Neuropsiquiatr 2022; 80: 13-22.
3) Hostettler IC, Seiffge DJ, Werring DJ. Intracerebral hemorrhage: an update on diagnosis and treatment. Expert Rev Neurother 2019; 19: 679-694.
4) Yan XJ, Li YB, Liu W, Dai WM, Wang CL. Predictive value of serum visinin-like protein-1 for early neurologic deterioration and three-month clinical outcome in acute primary basal ganglia hemorrhage: A prospective and observational study. Clin Chim Acta 2022; 531: 62-67.
5) Sun PY, Poblete RA, Nguyen PL, Bulic SF, Kim-Tenser MA, Marehbian J, Cen SY, Emanuel BA. Association Between Glycemic Gap and In-hospital Outcomes in Aneurysmal Subarachnoid Hemorrhage. Front Neurol 2021; 12: 714341.
6) Kraut JA, Madias NE. Serum anion gap: its uses and limitations in clinical medicine. Clin J Am Soc Nephrol 2007; 2: 162-174.
7) Martha SR, Collier LA, Davis SM, Seifert HA, Leonardo CC, Ajmo Jr CT, Foran EA, Fraser JF, Pennypacker KR. Translational Evaluation of Acid/Base and Electrolyte Alterations in Rodent Model of Focal Ischemia. J Stroke Cerebrovasc Dis 2018; 27: 2746-2754.
8) Liu X, Feng Y, Zhu X, Shi Y, Lin M, Song X, Tu J, Yuan E. Serum anion gap at admission predicts all-cause mortality in critically ill patients with cerebral infarction: evidence from the MIMIC-III database. Biomarkers 2020; 25: 725-732.
9) Baharoglu MI, Brand A, Koopman MM, Vermeulen M, Roos Y. Acute Management of Hemostasis in Patients With Neurological Injury. Transfus Med Rev 2017; 31: 236-244.
10) Brouwers HB, Goldstein JN, Romero JM, Rosand J. Clinical applications of the computed tomography angiography spot sign in acute intracerebral hemorrhage: a review. Stroke 2012; 43: 3427-3432.
11) Kotake Y. Unmeasured anions and mortality in critically ill patients in 2016. J Intensive Care 2016; 4: 45.
12) Vincent JL, Quintairos ESA, Couto L, JR., Taccone FS. The value of blood lactate kinetics in critically ill patients: a systematic review. Crit Care 2016; 20: 257.
13) Glasmacher SA, Stones W. Anion gap as a prognostic tool for risk stratification in critically ill patients - a systematic review and meta-analysis. BMC Anesthesiol 2016; 16: 68.
14) Chen Q, Chen Q, Li L, Lin X, Chang SI, Li Y, Tian Z, Liu W, Huang K. Serum anion gap on admission predicts intensive care unit mortality in patients with aortic aneurysm. Exp Ther Med 2018; 16: 1766-1777.
15) Yang SW, Zhou YJ, Zhao YX, Liu YY, Tian XF, Wang ZJ, Jia DA, Han HY, Hu B, Shen H, Gao F, Wang LY, Lin J, Pan GZ, Zhang J, Guo ZF, Du J, Hu DY. The serum anion gap is associated with disease severity and all-cause mortality in coro-

nary artery disease. J Geriatr Cardiol 2017; 14: 392-400.

16) Morgan AD, Sharma C, Rothnie KJ, Potts J, Smeeth L, Quint JK. Chronic Obstructive Pulmonary Disease and the Risk of Stroke. Ann Am Thorac Soc 2017; 14: 754-765.

17) de Miguel-Diez J, Lopez-de-Andres A, Jimenez-Garcia R, Hernández-c V, Jiménez-Trujillo I, Ji Z, de Miguel-Yanes JM, López-Herranz M. Sex Differences in the Incidence and Outcomes of COPD Patients Hospitalized with Ischemic Stroke in Spain: A Population-Based Observational Study. Int J Chron Obstruct Pulmon Dis 2021; 16: 1851-1862.

18) Sallinen H, Wu TY, Meretoja A, Putaala J, Tatlisumak T, Strbian D. Effect of baseline hypocalcaemia on volume of intracerebral haemorrhage in patients presenting within 72 hours from symptom onset. J Neurol Sci 2019; 403: 24-29.

19) Rajendran SR, Periyasamy S, Manjuladevi MT, George N. Evaluation of Serum Ferritin as a Prognostic Marker in Acute Hemorrhagic Stroke. J Neurosci Rural Pract 2020; 11: 72-77.