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Original Article

The correlation between noradrenaline and acetylcholine levels and autonomic nervous system dysfunction in patients with stroke-associated pneumonia

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Abstract: Background: This study was designed to detect plasma noradrenaline (NE) and acetylcholine (Ach) levels in patients with stroke-associated pneumonia (SAP), in order to analyze the correlation between SAP and autonomic nervous system dysfunction. Methods: A total of 300 patients diagnosed with acute stroke in our hospital from October 2014 to October 2016 were enrolled into this study. These patients were divided into two groups: pneumonia group and stroke group. In addition, a control group was also established (n=300). Baseline data of all subjects were collected. These subjects were rated using the autonomic symptom profile (ASP), and were detected for NE and Ach levels. Then, a statistical analysis was conducted. Results: Among these 300 stroke patients, 55 patients were combined with pulmonary infections; and the incidence was 18.3%. The independent risk factors for SAP were as follows: age ≥65 years, smoking history, lung disease history, stroke history, diabetes, coronary heart disease, atrial fibrillation, stroke type, Kubota's drinking water test, dysphagia, nasal feeding, National Institutes of Health Stroke Scale (NIHSS) score, Glasgow coma score, hypoproteinemia, bleeding volume, white blood cell count, and C-reactive protein. ASP score was higher in SAP patients than in stroke patients, NE level was higher in SAP patients than in stroke patients, and Ach level was lower in SAP patients than in stroke patients. Ach level was negatively correlated with ASP in patients with SAP (P<0.05), and NE level was positively correlated with ASP (P<0.05). Conclusion: Autonomic nervous system dysfunction may be one of the pathogenesis of SAP.

Keywords: Stroke associated pneumonia, norepinephrine, acetylcholine

Introduction

Acute stroke patients often develop many complications, and those with advanced age, have an infirm constitution, are long-term bedridden, have impaired lung function, and accompanied by impaired consciousness are more prone to stroke-associated pneumonia (SAP) [1]; and its incidence range between 7-22%. SAP is the main cause of poor prognosis and death in stroke patients [2-4].

Acute stroke is followed by sympathetic excitation, which increases catecholamine secretion. The latter can selectively inhibit the secretion of interferon-γ (IFN-γ) from Thl cells, and the administration of the β-adrenergic receptor blocker can increase the production of IFN-γ [5]. The study conducted by Sykom *et al*. revealed that adrenergic antagonists may improve immunosuppression and infections induced by sympathetic excitation after stroke [6]. Stimulation on vagal parasympathetic nerve modulates cerebral and systemic inflammatory responses release NA and activate the cholinergic anti-inflammatory pathway [7]. After stroke, stimulation on the nicotinic acetylcholine receptor reduces inflammation, protects neurons from oxidative stress and improves functional recovery [8]. The study conducted by Ottani *et al*. pointed out that in the later stage of stroke, melanocortin regulates the cholinergic

anti-inflammatory pathway and downregulates tumor necrosis factor [9], which leads to the occurrence of SAP. These findings suggest that autonomic nervous system dysfunction (ANSD) occurs after acute stroke, and ANSD may attenuate inflammation, reduce the immunity of stroke patients, and thereby induce SAP. The autonomic nervous system consists of two basic components: sympathetic and parasympathetic nervous systems. The neurotransmitter released from sympathetic postganglionic fibers is noradrenaline (NE), while the neurotransmitter released from parasympathetic postganglionic fibers is acetylcholine (Ach).

In the present study, plasma NE and Ach levels in patients with SAP were observed, and the correlation between these two and SAP was analyzed, in order to provide a theoretical basis for clinical application of autonomic nervous system drugs and prevent the occurrence of SAP.

General information

Subjects

A total of 300 patients admitted in the Neurology Department of our hospital, who were diagnosed with acute stroke from October 2014 to October 2016, were enrolled into this study. Among these patients, 213 patients had cerebral infarction, 10 patients had post-infarction hemorrhage, and 77 patients had cerebral hemorrhage. All patients met the diagnostic criteria established by the Fourth National Conference on Cerebrovascular Diseases. These patients were divided into two groups according to the presence of SAP: pneumonia group and stroke group. Among the 55 patients in the pneumonia group, 28 patients had cerebral infarction, six patients had post-infarction hemorrhage, and 21 patients had cerebral hemorrhage. Furthermore, among these 55 patients, 31 patients were male and 24 patients were female; and the age of these patients ranged between 61-85 years old, with an average age of 71.15±12.72 years old. Among these 245 patients in the stroke group, 185 patients had cerebral infarction, four patients had post-infarction hemorrhage, and 56 patients had cerebral hemorrhage. Furthermore, among these 245 patients, 156 patients were male and 89 patients were female; and the age of these patients ranged between 58-83 years old, with an average age of 68.46±10.29 years old. A control group was set (n=300), which comprised of 185 male and 115 female healthy subjects who underwent physical examination in the Physical Examination Center. The age of these subjects ranged between 60-80 years old, with an average age of 69.35±11.58 years old.

Inclusion criteria: (1) patients admitted within 72 hours after onset; (2) patients who developed stroke for the first time; (3) patients with stroke diagnosed by cranial computed tomography (CT) or magnetic resonance imaging (MRI); (4) patients who provided a signed informed consent, and were approved by the ethics committee of the hospital to participate in this study.

Exclusion criteria: (1) patients who had heart failure, hepatic and renal insufficiency, and chronic obstructive pulmonary disease; (2) patients who had taken drugs that could affect autonomic nervous function before testing such as hormones, adrenergic drugs and β-receptor blockers; (3) patients who were combined with other neurological disorders such as polyneuropathy and orthostatic hypotension; (4) patients who had infectious and latent diseases during the test such as pneumonia and urinary tract infection.

Diagnostic criteria for stroke-associated pneumonia

Clinical diagnostic criteria: New or progressive lesions were revealed by chest imaging after stroke. Patients who had two or more of the following clinical symptoms were excluded from the study: patients with a body temperature of ≥38°C; patients with cough, expectoration, exacerbated respiratory tract disease, or chest pain; patients with signs of pulmonary consolidation and (or) moist rales; patients with a white blood cell count $\geq 10 \times 10^9$/L or $\leq 4 \times 10^9$/L, with or without nuclear shift to the left; patients with tuberculosis, lung cancer and other similar diseases.

Etiological diagnostic criteria: In the early morning, patients first gargled with saline three times, coughed out the deep phlegm, and left sputum specimens in a sterile culture tube. For patients who had difficulties in doing this procedure, deep phlegm was sucked out using a

suction catheter, and was sent for testing within 30 minutes. The VITEK microbial identification system and the minimum inhibitory concentration method were used to determine drug sensitivity. When a large number of spores and hyphae were found in the smear, it was determined as positive for fungi.

Materials and methods

Data collection for all patients

(1) Baseline data: gender, age, smoking history, drinking history, and past history (including coronary heart disease, hypertension, cerebral infarction, diabetes, atrial fibrillation, and chronic bronchitis). (2) Clinical characteristics: location and area of infarction, degree of limb paralysis, consciousness disorders, dysphagia, cough, and expectoration. (3) Adjuvant examinations: blood glucose, blood lipids, homocysteine, C-reactive protein, hemoglobin, albumin, and white blood cell count. (4) Others: auxiliary treatment measures such as nasal feeding, endotracheal intubation, etc.

Patients were scored according to the autonomic symptom profile

A score ≤22 points indicates normal and a score >22 points indicates abnormal. The higher the score was, the more serious the ANSD become.

NE detection

For all patients, 3 ml of fasting venous blood was collected on the second day after admission to the hospital. The same amount of fasting venous blood was collected in the early morning at the rest state for healthy subjects. All blood samples were stored in pre-cooled anticoagulant tubes. Blood samples underwent low temperature centrifugation at 4°C (1,500 rpm for 15 minutes), and the supernatant was obtained and preserved at a low temperature of -80°C. Plasma NE level was detected using liquid chromatography-mass spectrometry. (1) Chromatographic conditions: The chromatographic column was the ZORBAX SB-Aq column; mobile phase A was 0.08% formic acid and mobile phase B was acetonitrile; between the 0 and 8th minute, phase A was maintained at 95%, and phase B was maintained at 5%; afterwards, phase B rose to 70% and remained constant; between the 14th and 15th minute, phase B decreased to 5%, phase A remained at 95%, and the flow rate was set at 0.5 ml/min. (2) Mass spectrometry conditions: An electrospray ion source was used. Positive ion mode was selected, dry gas flow rate was 12 L/min, temperature was 280°C, atomization pressure was 45 psi, and capillary voltage was 4,000 V. The scanning mode was the multiple reaction monitoring mode (MRM).

Ach level detection

Plasma Ach levels in all subjects were measured by enzyme-linked immunosorbent assay (ELISA), and the standard curve was drawn (with the standard concentration as the abscissa and the optic density [OD] value as the ordinate). According to the OD values, the concentrations of the samples were calculated using the standard curve regression equation and results were multiplied by the corresponding dilution times. Thus, the actual concentrations of the sample were obtained.

Statistical analysis

Data were statistically analyzed using statistical software SPSS 19.0. Normally distributed measurement data were expressed as mean ± standard deviation (mean ± SD), and were evaluated using *t*-test. Counting data were expressed as case (%) and evaluated using Chi-square test. Variables were screened by logistic univariate analysis. Then, multivariate logistic regression analysis was performed to determine risk factors. $P<0.05$ was considered statistically significant.

Results

Mortality comparison

Among these 300 stroke patients, a total of 55 patients were combined with pulmonary infections, and the incidence was 18.3%. Among the 55 patients in the pneumonia group, 12 patients died; and the mortality rate was 21.8%. Among the 245 subjects in the control group, eight subjects died; and the mortality rate was 3.27%. The difference in mortality rate between these two groups was statistically significant ($P<0.01$).

*Comparison of risk factors between the pneumonia group and stroke group (**Table 1**)*

The univariate analysis of risk factors in these two groups suggest that differences in the number of patients ≥65 years old, smoking his-

Table 1. Comparison of risk factors between the pneumonia group and stroke group

Risk factors	Pneumonia group (n=55)	Stroke group (n=245)	t/x^2	*P*-values
Age			28.139	0.000
≥65 years old	39	79		
<65 years old	16	166		
Gender			1.022	0.312
Male	31	156		
Female	24	89		
Smoking history	16	32	8.587	0.003
Drinking history	10	45	0.001	0.974
Lung disease history	20	37	13.194	0.000
Stroke history	16	37	6.043	0.015
Diabetes	12	23	10.696	0.001
Hypertension	42	201	0.941	0.332
Coronary heart disease	9	95	9.961	0.002
Atrial fibrillation	18	35	10.502	0.001
Stroke type			8.478	0.014
Hematencephalon	21	56		
Cerebral infarction	32	181		
Post infarction hemorrhage	4	6		
Kubota's drinking water test			21.594	0.000
≥level 3	31	60		
<level 3	24	185		
Dysphagia	18	28	15.695	0.000
Nasal feeding	40	72	36.061	0.000
NIHSS score	15.42±6.33	7.65±3.21	12.531	0.002
<6 points	22	161		
6~13 points	23	60		
≥14 points	10	24		
Hypoproteinemia (<25 g/L)	27	53	17.317	0.000
GCS score	6.05±2.68	4.39±2.17	9.842	0.011
Bleeding volume	24.12±5.64	17.44±3.62	11.295	0.001
Total cholesterol (mmol/l)	5.23±1.06	4.23±1.25	0.078	0.875
Low density lipoprotein (mmol/L)	3.04±0.88	2.56±1.12	0.053	0.962
White blood cell count	12.82±1.35	8.73±1.94	18.38	0.000
Homocysteine (μmol/l)	15.51±12.66	13.8±13.51	0.112	0.549
C-reactive protein (mg/L)	4.86±5.32	2.48±0.53	6.217	0.033
Fibrinogen (g/L)	3.59±0.87	3.05±0.99	0.093	0.641

tory, lung disease history, diabetes, coronary heart disease, atrial fibrillation, Kubota's drinking water test, dysphagia, nasal feeding, National Institutes of Health Stroke Scale (NIHSS) score, hypoproteinemia, bleeding volume and white blood cell count between these two groups were statistically significant (*P*<0.01). Furthermore, differences in stroke history, Glasgow coma score (GCS), stroke type and C-reactive protein were statistically significant (*P*<0.05). Moreover, differences in gender, hypertension, drinking history, total cholesterol, low density lipoprotein, homocysteine and fibrinogen were not statistically significant (*P*>0.05).

*Multivariate analysis of common risk factors in patients with pneumonia (**Table 2**)*

Variables with statistical significance in the univariate analysis were included in the multivariate logistic regression analysis. Results revealed that age ≥65 years old, smoking history, lung disease history, stroke history, diabetes, coronary heart disease, atrial fibrillation, stroke type, Kubota's drinking water test, dysphagia, nasal feeding, NIHSS score, Glasgow coma score, hypoproteinemia, bleeding volume, white blood cell count and C-reactive protein could be included into the regression equation. Furthermore, these results revealed that these factors were all independent risk factors for SAP.

*ASP scores, NE and Ach detection results in the three groups (**Table 3**)*

Among these three groups, the pneumonia group had the highest ASP score, the difference was statistically significant compared with the stroke group, and the difference between the stroke group and control group was statistically significant. Among these three groups, the pneumonia group had the highest Ach level, the difference was statistically significant compared with the stroke group, and the difference between the stroke group and the control group was statistically significant. Among the three

Table 2. Multivariate analysis of common risk factors in patients with pneumonia

Risk factors	OR	95% CI	*P*-values
≥65 years old	2.94	1.65-6.83	0.007
Smoking history	2.38	1.14-3.82	0.009
Lung disease history	3.68	2.23-4.16	0.000
Stroke history	2.31	2.02-3.97	0.009
Atrial fibrillation	2.63	2.31-4.27	0.000
Diabetes	2.54	1.77-3.86	0.008
Coronary heart disease	2.13	1.23-21.87	0.010
Stroke type	1.79	1.58-2.23	0.013
Kubota's drinking water test	4.77	3.89-6.39	0.000
NIHSS score	2.28	1.23-4.94	0.009
Dysphagia	15.55	4.08-10.77	0.000
Bleeding volume	3.21	1.14-1.78	0.006
Nasal feeding	15.47	8.59-32.18	0.000
GCS score	5.47	3.69-8.62	0.000
Hypoproteinemia	3.87	1.06-3.98	0.000
White blood cell count	1.52	1.18-1.83	0.019
C-reactive protein	1.23	1.05-2.09	0.026

Table 3. ASP scores, NE and Ach detection results in the three groups

	Pneumonia group (n=55)	Stroke group (n=245)	Control group (n=300)
ASP (points)	32.47±6.85Δ,#	26.89±5.13*	15.26±4.39
Ach (ng/L)	60.28±13.26Δ,#	74.56±12.17*	111.64±15.25
NE (ng/mL)	2.07±0.89Δ,#	1.71±0.68*	0.58±0.16

Δ: Compared with the stroke group P<0.01; #: Compared with control group P<0.01; *: Compared with control group P<0.01.

Table 4. Correlation between plasma Ach and NE levels and ASP scores in SAP patients

Parameter	r	P
ASP and Ach	-0.0768	0.012
ASP and NE	0.743	0.041

groups, the pneumonia group had the highest NE level, the difference was statistically significant compared with the stroke group, and the difference between the stroke group and the control group was statistically significant.

*Correlation between plasma Ach and NE levels and ASP scores in SAP patients (**Table 4**)*

In SAP patients, Ach level was negatively correlated with ASP ($P<0.05$), and NE level was positively correlated with ASP ($P<0.05$).

*Comparison of plasma NE and Ach levels and the incidence of SAP among the three groups (**Table 5**)*

The incidence of pneumonia in patients with post-infarction hemorrhage was the highest, the differences were statistically significant compared with cerebral infarction patients and cerebral hemorrhage patients, and the difference between cerebral hemorrhage patients and cerebral infarction patients was statistically significant. The NE level in patients with post-infarction hemorrhage was the highest, the differences were statistically significant compared with cerebral infarction patients, cerebral hemorrhage patients and healthy subjects, the differences between the stroke group and cerebral hemorrhage group, and between the stroke group and control group were statistically significant, and the difference between the cerebral hemorrhage group and control group was statistically significant. The Ach level in patients with post-infarction hemorrhage was the lowest, the differences were statistically significant compared with cerebral infarction patients, cerebral hemorrhage patients and healthy subjects, the differences between the cerebral infarction group and cerebral hemorrhage group, and between the cerebral infarction group and control group were statistically significant, and the difference between the cerebral hemorrhage group and control group was statistically significant.

Discussion

SAP is one of the common complications of acute stroke. This not only affects the recovery of neural function, but also significantly increases the mortality of patients [1]. Many studies have suggested that the occurrence of SAP is associated with the following risk factors: age [3], smoking history [4], stroke severity [3], the degree of paralysis [10], dysphagia [11], nasal feeding [12], basal lung disease [13], diabetes [14], atrial fibrillation [14], hypertension [15], infarct size [16], disturbance of consciousness and hypoproteinemia; and these were consistent with the results of this study. This study

Table 5. Comparison of plasma NE and Ach levels and the incidence of SAP among the three groups

Groups	Incidence rate (%)	NE	Ach
Cerebral infarction (n=213)	15.02 (32/213)$^{\#,*}$	1.92±0.36$^{\#}$	69.37±12.69$^{\#,*}$
Cerebral hemorrhage (n=77)	27.27 (21/77)$^{\Delta}$	1.73±0.24$^{\#,\$}$	76.56±14.57$^{\#,\$}$
Post-infarction hemorrhage (n=10)	40 (4/10)	2.16±0.42$^{\#}$	67.83±11.22$^{\#}$
Control group (n=300)		0.58±0.16	111.64±15.25

Compared with the incidence of SAP: #: Compared with control group P<0.01; *: Compared with post-infarction hemorrhage group P<0.01; Δ: Cerebral hemorrhage compared with post-infarction hemorrhage group P<0.01. NE compared with Ach levels: #: Compared with cerebral hemorrhage group P<0.01; *: Compared with cerebral hemorrhage group P<0.05; Δ: Compared with cerebral infarction P<0.01; $: Compared with post-infarction hemorrhage group P>0.05.

also revealed that age ≥65 years old, smoking history, lung disease history, stroke history, diabetes, coronary heart disease, atrial fibrillation, stroke type, Kubota's drinking water test, dysphagia, nasal feeding, NIHSS and GCS scores, hypoproteinemia, bleeding volume, white blood cell count and C-reactive protein were independent risk factors for SAP. The main reasons of its pathogenesis are as follows: (1) Elderly patients often mostly have organ hypofunction, decreased immunity, and concurrently develop multiple basal diseases [17]. (2) The lung volume, reserve capacity of respiratory function, diffusing capacity, ability to clear the secretion, and defense ability of elderly patients decrease and result in decreased pulmonary ventilation. (3) A hyperglycemia environment is propitious to the growth and propagation of bacteria, and is likely to cause bacterial infection. Hyperglycemia can decrease the phagocytic ability of white blood cells and macrophages, and decrease the resistance of the body to bacteria. Hyperglycemia can also cause slow blood flow, and decrease the oxygen carrying capacity of hemoglobin, which easily induces pulmonary congestion [18]. (4) After acute stroke, cough reflex decreases, and the sputum and secretions could not be easily coughed out. (5) For patients with swallowing dysfunction, nasopharyngeal secretions and food residue can easily to be inhaled into the lungs, which contain a large number of pathogenic microorganisms, causing the pathogen load to reach a certain extent, thereby leading to pneumonia [19]. (6) The local defense function of the respiratory tract decreases. Hence, vasoactive substances easily lead to lung injury, and damage the alveolar epithelium. Under this condition, cytokines can promote the accumulation of inflammatory cells, inflammatory mediators are released, and finally susceptibility increases. (7) Compensatory hyperfunction in the peripheral immune system causes immune suppression, and may induce SAP [20]. (8) Patients with acute stroke will develop hypothalamic-pituitary-adrenal dysfunction, their immunity decreases, lymphocyte reduces, and tumor necrosis factor concentration decreases, which increases the risk of pneumonia. In summary, SAP is caused by various risk factors on the basis of immune suppression syndrome in the early stage of acute stroke.

In the present study, ASP score was higher in SAP patients than in stroke patients. This suggests that pneumonia patients were combined with severe ANSD. The ASP score in patients with stroke was higher than in controls, which suggests that acute stroke patients were combined with ANSD, manifested as an accelerated heart rate and increased blood pressure, ecphysesis, hyperhidrosis, and inhibited organ and tissue secretion. These were consistent with related studies [21, 22].

NE level was higher in SAP patients than in stroke patients, and the difference was statistically significant. These results suggest that the sympathetic activity in patients with pneumonia was stronger than in patients with stroke. In addition, plasma Ach level in patients with pneumonia was lower than in patients with stroke, and the difference was statistically significant. These results suggest that the parasympathetic activity in patients with pneumonia was weaker than in patients with stroke. Plasma NE level increased and Ach level decreased in patients with pneumonia. These results revealed that pneumonia patients were combined with ANSD, and this was highly consistent with the ASP scores in patients in the two groups. These two results both revealed that pneumonia patients were combined with severe ANSD. Furthermore, the ASP score was positively correlated with NE levels and nega-

tively correlated with Ach levels. A study conducted by Kuriyama *et al*. [23] revealed that acute supratentorial cerebral infarction revealed decreased parasympathetic regulation and accordingly increased sympathetic nerve output. Acute stroke is often accompanied by obvious symptoms of autonomic nerve, and most of these symptoms were caused by damage to the hypothalamus directly and indirectly induced by lesions [24]. Lahuz-Roszak *et al*. [25] confirmed in their study that after stroke, patients were combined with dysfunction of tje sympathetic and parasympathetic system. The reason is the injuries in central autonomic neural network, especially in the cortical frontal-parietal area and brain stem, or interruption of the autonomic nerve conduction path [23]. This study revealed that acute stroke patients were combined with ANSD, which may induce SAP. Animal experiments have also confirmed that propranolol decreased the apoptosis of white blood cells by reducing the excitability of sympathetic nerves, thereby reducing the incidence of SAP [26]. The mechanism may be as follows: (1) Sympathetic excitation increases plasma catecholamine content, causes the contraction of the vascular system of the systemic circulation, and increases pulmonary circulation pressure, allowing a large amount of body fluid to gather between the tissues of the lung, thereby causing pulmonary edema. In addition, the pulmonary vein contracts and capillary pressure increases. This in turn causes damage to endothelial cells, increase vascular permeability, and induce permeability pulmonary edema. (2) Persistent sympathetic excitation leads to pulmonary edema and hypoxia. Hence, it impairs the immune function and cleaning ability of the local respiratory tract, and impairs the phagocytic ability of white blood cells [27]. (3) After acute stroke, the hypothalamic-pituitary-adrenal axis (HPA) becomes active, causes ANSD, and increases glucocorticoid secretion. At the same time, glucocorticoids can inhibit the production of pro-inflammatory mediators, stimulate anti-inflammatory mediators to release [28], and inhibit the immune function of the body by inducing the apoptosis of T lymphocytes [29]. (4) After acute stroke, excessive excitation of the HPA axis and sympathetic system is induced, which affects the immune system, reduces immune cells, induce function decline and changes in cytokine levels, and induce immune deficiency syndrome [30]. (5) Walter *et al*. [31] confirmed through a clinical study that after cerebral infarction, the levels of epinephrine, NE and neutrophils in blood increased, and T lymphocyte count decreased; and these were proportional to the area of cerebral infarct. Indeed, there are also many mechanisms of SAP such as increased Th1 cells and their cytokines IFN-γ in early stage of cerebral infarction, while patients with severe cerebral infarction show a downward trend a week after cerebral infarction [32]. This also explains that SAP often occurs in patients with severe stroke.

This study also revealed that the plasma NE level in patients with post-infarction hemorrhage was higher than in patients with cerebral infarction and patients with cerebral hemorrhage, while Ach level was lower than in patients with cerebral infarction and patients with cerebral hemorrhage. These results suggest that among these three groups, patients in the post-infarction hemorrhage group had the highest sympathetic activity, the lowest parasympathetic activity, the most serious ANSD, and the highest incidence of SAP. It is possible that post-infarction hemorrhage is usually caused by the large area of cerebral infarction. Patients with post-infarction hemorrhage had higher plasma NE level than patients with cerebral hemorrhage, and lower Ach level than patients with cerebral hemorrhage. This suggests that patients with cerebral infarction had higher sympathetic activity and lower parasympathetic activity compared with patients with cerebral hemorrhage. The reason may be that the insular lobe is the center of the autonomic nervous system, and intracerebral hemorrhage is mainly located in the basal ganglia. Another result of this study revealed that the incidence of SAP was higher in patients with cerebral hemorrhage than in patients with cerebral infarction, which was not inconsistent with the incidence of ANSD. The reason may be that cerebral hemorrhage patients are often maintain in the lying position, and being bedridden for a long time would make it difficult for respiratory tract secretions to be discharged, which would eventually accumulate at the lower sites, and increase the incidence of SAP. In addition, patients with cerebral hemorrhage have more serious conditions, and are more likely to receive invasive operations and dehydration drugs. These also reveal that the pathogenesis of SAP is complex, and that ANSD may be one

of the pathogenesis of SAP, but not the main mechanism.

In summary, this study revealed that SAP is caused by many factors. Amid clinical practices, we should take effective preventive measures for different risk factors. We can also control plasma NE and Ach levels by drugs, in order to reduce the occurrence of ANSD, reduce the incidence of SAP as far as possible, shorten hospitalization time, and improve the prognosis of patients with acute stroke.

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Disclosure of conflict of interest

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