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Early predictors of brain injury in patients with acute carbon monoxide poisoning and the neuroprotection of mild hypothermia

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ABSTRACT

Introduction: Carbon monoxide (CO) poisoning can cause serious neurological sequelae. However, there is neither effective treatment strategy nor reliable indicators to determine the prognosis of patients with CO poisoning. The present study aimed to observe the changes of neurological function score, disease severity score, cerebral oxygen utilization (O_2UCc), bispectral (BIS) index and neuron-specific enolase (NSE) concentration, and to elucidate the clinical significance of these potential indicators and the neuroprotective effect of mild hypothermia on brain injury in patients with severe acute CO poisoning.

Materials and methods: A total of 277 patients with acute severe CO poisoning from 2013 to 2018 were enrolled in our hospital. Patients were divided into three groups according to their body temperature on the day of admission and their willingness to treat: a fever group ($n = 78$), a normal temperature group (NT group, $n = 113$), and a mild hypothermia group (MH group, $n = 86$). All patients were given hyperbaric oxygen therapy, while those in the MH group received additional mild hypothermia treatment. The severity of the disease, the neurobehavioral status, the incidence of delayed encephalopathy after acute carbon monoxide poisoning (DEACMP), and other indicators including BIS, O_2UCc, NSE were further evaluated in all patients at given time-points.

Results: Mild hypothermia therapy improved the prognosis of patients with CO poisoning, significantly decreased the value of O_2UCc and NSE, and up-regulated BIS. The incidence of DEACMP at 6 months was 27% in the fever group, 23% in the NT group, and 8% in the MH group. The values of Glasgow-Pittsburgh coma scale (G-P score), BIS index and NSE were closely related to the occurrence of DEACMP, the cutoff values were 12.41, 52.17 and 35.20 ng/mL, and the sensitivity and specificity were 79.3%, 77.6%, 79.3% and 67.6%, 89.5%, 88.6% in the receiver operating characteristic curve (ROC), respectively.

Conclusions: Early mild hypothermia treatment could significantly reduce the severity of brain injury after CO poisoning, and might be further popularized in clinic. G-P scores, NSE and BIS index can be regarded as the prediction indicators in the occurrence and development of DEACMP.

Clinical trial registration: The study protocol was granted from Qingdao University Research Ethics Committee (Clinical trial registry and ethical approval number: QD81571283).

1. Introduction

Carbon monoxide (CO) is choking gas that has been regarded as venenation of respiratory enzyme hemoglobin. Delayed encephalopathy after acute CO poisoning (DEACMP) is one of the most serious sequelae, and may occur in some patients after the acute phase clinical symptom was remission with medical help. The incidence, the mortality and disability rate of the disease are very high in severe CO poisoning patients. Therefore, early treatment and protection of nervous system are very important for prognosis and life quality of patients with CO poisoning.

Mild hypothermia is a treatment strategy to reduce the patient's core temperature at 32 °C ~ 35 °C. It can help patients survive the

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acute phase of brain injury and preserve neurological function [1]. Research shows mild hypothermia can reduce cell metabolic rate, inhibit the production of free radicals and inflammatory factors during stress. Up to now, it has been applied in the treatment of cerebral infarction and CO poisoning by many experts [2,3]. However, most of them are case reports or retrospective studies [4,5].

Bispectral (BIS) index can quickly and accurately reflect the function of cerebral cortex. Cerebral oxygen utilization coefficient (O_2UCc) can reflect the relationship between cerebral blood perfusion and cerebral metabolism, and the state of cerebral blood circulation. Neuron-specific enolase (NSE) is also closely associated with the extent of brain damage. However, it is not clear whether the above indicators can be used to determine the prognosis of brain injury induced by CO poisoning. The present study systematically observed the changes of neurological function score, disease severity score, O_2UCc, BIS index and NSE concentration, elucidated the clinical significance of these indicators for the prognosis of brain injury in acute severe CO poisoning patients, clarifing the efficacy and safety of mild hypothermia in the treatment of CO poisoning.

2. Materials and methods

2.1. Study design and patients

This observational study was conducted in Yantai Yuhuangding hospital affiliated to Qingdao University from January 2013 to December 2018. A total of 312 patients were hospitalized within 24 h after poisoning and met diagnostic criteria for acute severe CO poisoning [6] with concentration of carboxyhemoglobin (COHb) protein >40% and disturbance of consciousness. In the present study, all candidates regained consciousness within 24 h after treatment, and then completed all examination, treatment programs and follow-up plans.

Exclusion criteria were as follows: (1) patients with a medical history of neuropsychiatric illness, cognitive impairment, severe respiratory failure, heart failure, chronic obstructive pulmonary disease, severe liver and kidney dysfunction, severe immune system disease, cardio-cerebrovascular disease, malignant tumor, radioactive encephalopathy, encephalitis, multiple sclerosis, sarcoidosis and endocrine and metabolic diseases; (2) patients with drug abuse, food or other substances poisoning; (3) patients younger than 18 or older than 80; (4) patients with absolute contraindications of hyperbaric oxygen therapy (HOT) [7]; (5) midway death due to complications of other diseases, loss of contact, or withdrawal from the follow-up plan. As a result, 35 patients were excluded from the initial list, and the rest 277 cases were included in the statistical analysis. The procedure of patient enrollment and exclusion was shown in Fig. 1.

Then, according to their body temperature on the day of admission and their willingness to treat, patients who were willing to receive mild hypothermia treatment were classified into a mild hypothermia group (MH group, $n = 86$), and those with normal rectal temperature and unwilling to receive mild hypothermia treatment were assigned to a normal body temperature group (NT group, $n = 113$), while patients who refused mild hypothermia therapy with high rectal temperature above 38 °C were appointed to the fever group ($n = 78$).

All patients enrolled in the present study were hospitalized for treatment in accordance with the guidelines for clinical conventional treatment of CO poisoning, including continuous oxygen inhalation through nasal catheter, treatment of brain edema, promotion of brain cell metabolism, and improvement of microcirculation. The administration was involved in Mannitol, Citicoline, Oxiracetam, Adenosine triphosphate, Cytochrome C, and Coenzyme A. The subjects with fever were given antibiotics, physics or drugs to bring down the temperature if necessary. All patients without contraindications of HOT were given hyperbaric oxygen treatment and did not require mechanical ventilation. The other four patients with chronic obstructive pulmonary disease with type II respiratory failure (one in the fever group, two in the NT group and another one in the MH group), a relative contraindication of hyperbaric oxygen treatment [7], were firstly received ventilator assisted treatment, and then immediately assigned to receive HOT within 24 h, and included in the final category of statistics. Hyperbaric oxygen therapy parameters were set as follows: the treatment pressure was 2.4 ATA, pressurized for 20 min, and stabilized oxygen inhalation for 60 min, rested for 5 min, decompressed for 20 min for the first time, then the treatment pressure was 2.0 ATA. Each patient received hyperbaric oxygen therapy once a day for 10 consecutive days, and then rest for 3 days for a total of 30 times. Patients in the MH group were controlled their temperature with a medical temperature controller (model number: ZLJ-2000I, Antai electronic products Co., Ltd., Changchun, China). Briefly, mild hypothermia therapy was performed within 24 h after CO poisoning and then the temperature target was set at 34 °C ~ 35.5 °C. The target induction time of low temperature was 2 h, and the target duration of low temperature was maintained for 5 days. The technology of active temperature control was adopted to reheat slowly, and the heating rate was 1 °C per 12 h (Fig. 2). The vital signs of each patient were closely monitored during mild hypothermia treatment. If the patients had chills and shivers due to low temperature, pethidine hydrochloride (loading dose: 1 mg/kg, maintenance dose: 25–45 mg/h) and midazolam (loading capacity: 0.1 mg/kg, maintenance dose: 2–6 mg/h) should be given in combination. Treatment strategies of the three groups were shown in Fig. 3.

2.2. Severity assessment of disease

According to the criteria of Table 1 and Akavipat et al. [8], G-P score and APACHE II score were performed in all patients by two professionals in the neurologic intensive care unit at the same time quantum (9 a.m.) before treatment and 1 day, 3 days, 7 days, 1 month after treatment. Then the changes of these scale values were determined and compared among the three groups by two trained raters in a double blind manner at the same time point.

2.3. Neurobehavioral score

Hasegawa Dementia Scale (HDS) and mini mental status examination (MMSE) were used to evaluate the neurobehavioral changes by two neurological professional staffs before treatment and 7 days, 1 month and 6 months after treatment simultaneously in a double-blind manner, and the neurobehavioral scores were calculated and compared among the three groups.

2.4. BIS index dynamic monitoring

The detection process of BIS index was carried out in strict accordance with the instructions of the bispectral index (BIS™) monitor (Covidien, Boulder, CO, Norwood, MA, USA). This monitor was a quantitative EEG device that uses a proprietary algorithm to analyze the electrical signal derived from a frontal electrode array to generate a number between 0 and 100. The BIS values >80 indicate that the patient was awake, while the values between 60 and 80 indicated sedation that the patient may respond purposefully to stimulus, and the values between 40 and 60 were considered to be reflect a level of unconsciousness suitable for surgery [9]. BIS index was recorded at 3 independent time points (9:00, 10:00, and 11:00 a.m., respectively) for each patient at 1 day, 3 days, 7 days, and 1 month, respectively. The average value was expressed as the BIS indexes of the day. Patients did not take sedation, analgesia, hibernation, muscle relaxants and other drugs as far as possible to avoid drug influence during dynamic monitoring. If the patient used the above

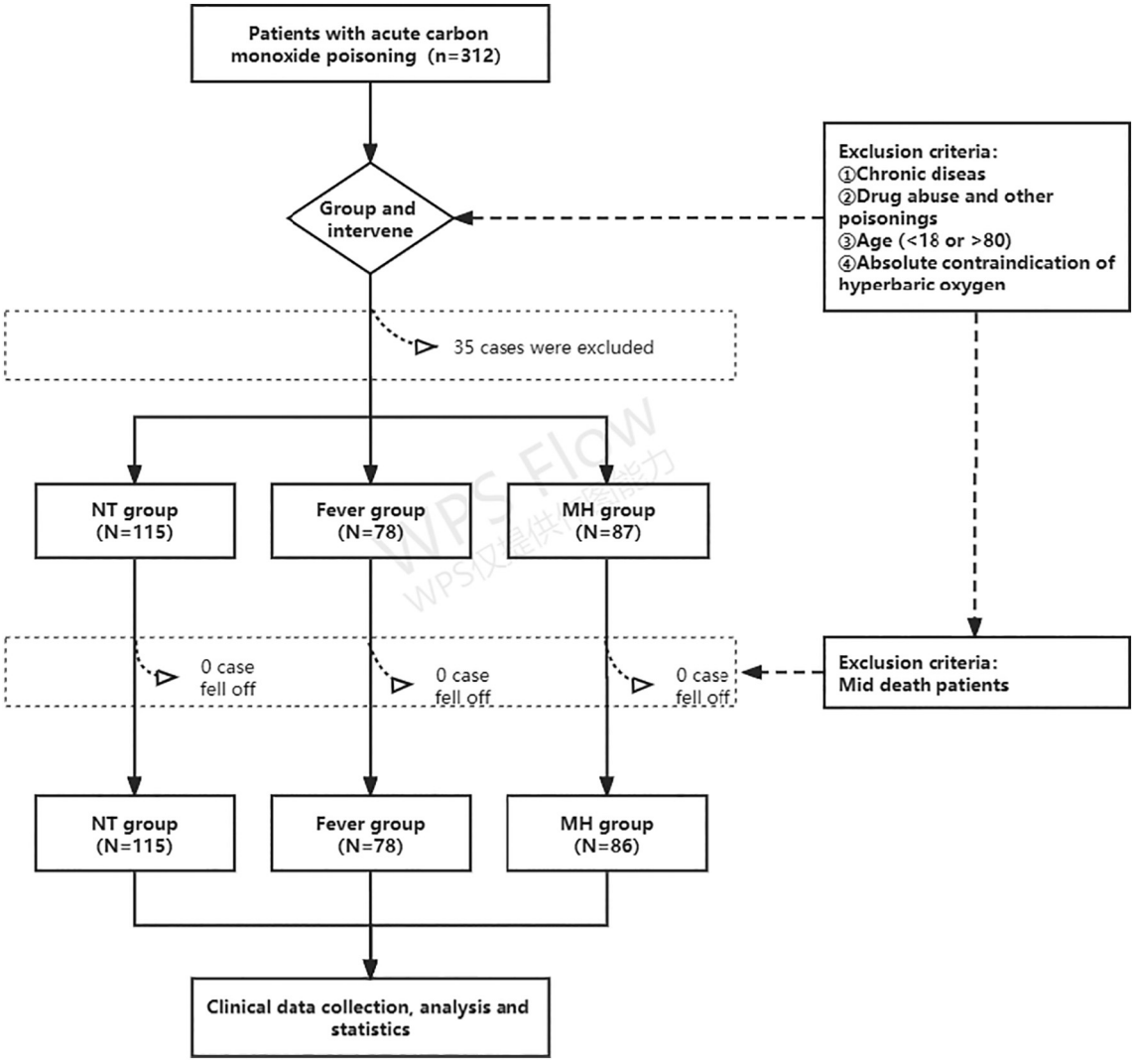

Fig. 1. The selection procedure of patient enrollment and exclusion.

drugs due to convulsions or agitation, the monitoring values should be observed 3 h to 4 h after discontinuation.

2.5. Detection of NSE concentration

A total of 3 mL fasting venous blood was collected from all the patients at 9:00 a.m. at the given time-points above. The serum was separated after centrifugation for the detection. NSE concentration was carried out in strict accordance with the operation instructions of the Abbott Architect i2000-SR® automatic immuno-analyzer by two trained raters in a double blind manner at the same time-point.

2.6. Changes of cerebral oxygen utilization coefficient (O_2UCc)

Blood gas analysis was performed in accordance with the instructions using GEM Premier 3000 blood gas analyzer (USA). A total of 2 mL blood samples were collected from the right internal jugular vein and radial artery before treatment and 1 day, 3 days, 7 days and 1 month after treatment. Oxygen saturation in radial artery (SaO_2) and internal jugular vein (SvO_2) was measured by two independent professionals in a double blind manner at the same time-point. The results of O_2UCc were calculated and expressed according to the formula: $O_2UCc = SaO_2 - SvO_2/SaO_2$.

2.7. Outcomes and prognosis judgment

The outcomes and prognosis of the three groups were collected by two professionals at 6 months after treatment, including the incidence of delayed encephalopathy and other serious neurological sequelae (language, activity ability), and the average duration of coma. The occurrence time of DEACMP and other neurological sequelae were taken as survival analysis.

2.8. Statistical analysis

SPSS 19.0 statistical software was used in the present study. The measurement data were expressed as mean ± S.D. (standard deviation). The repeated measurement analysis of variance (F test) was applied in the comparison among multiple groups, and then the mean of the measurement data between the two groups were compared with the least significant difference (LSD) test. Chi square test (χ^2 test) was used to compare the qualitative data. For some small values, especially $N < 10$, Fisher exact probability method was used. The Kaplan-Meier was used for survival analysis and the Logistic regression analysis was used to calculate the relative odds ratio (OR). Finally, the receiver operating characteristic curve (ROC) and the area under the curve (AUC) were analyzed the diagnostic efficacy and threshold values of G-P, BIS and NSE. $P < 0.05$ was statistically significant.

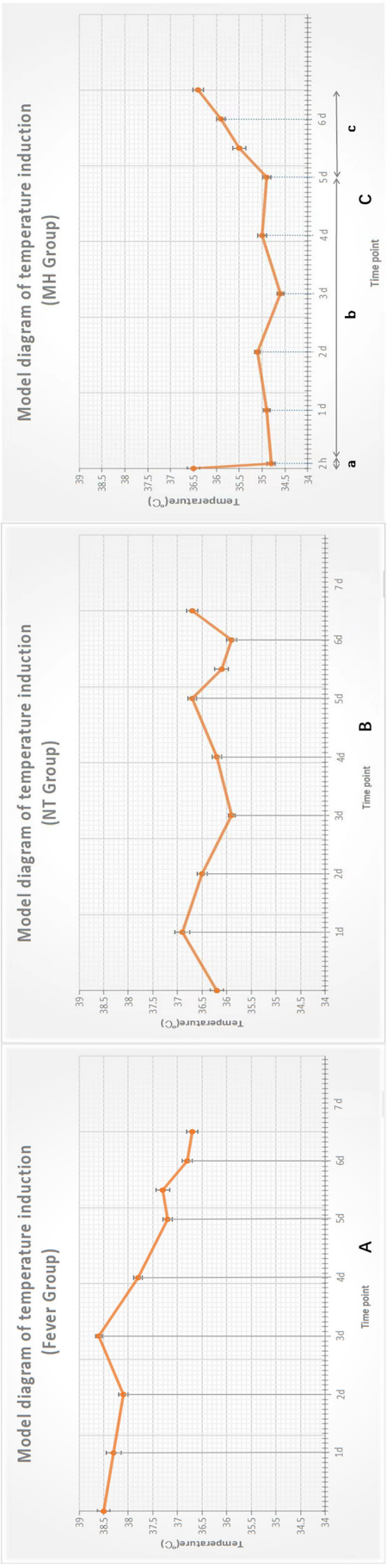

Fig. 2. Temperature trend chart of the three groups.
A: Fever group; B: NT group; C: MH group.
a, Target induction time; b, Duration of MH; c, Resuscitation time.

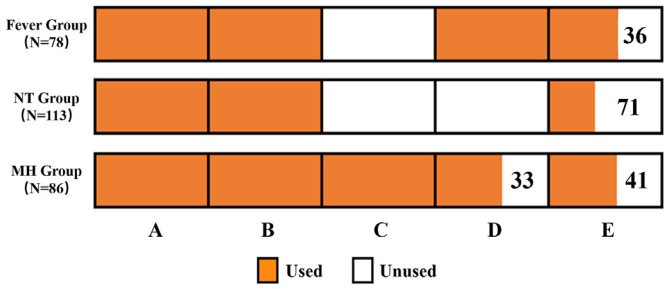

Fig. 3. Treatment of enrolled patients.
A: Hyperbaric oxygen therapy, **B:** Conventional therapy, **C:** Mild hypothermia treatment, **D:** Antimicrobial therapy, **E:** Other therapy.

3. Results

3.1. Comparison of general information

No significant differences were found among the three groups in age, gender composition, time from poisoning to visit hospital, occupation, education, COHb concentration, end-point oxygen saturation, blood glucose, coma time, number of patients receiving mechanical ventilation or GCS-Pittsburgh score (G-P score), APACHE II score, BIS index, and NSE concentration at first admission (Table 2, $P > 0.05$).

Table 1
Glasgow-Pittsburgh Coma Score, GCS-P.

No.	Items	Score
1.	**Best Eye Response**	
	Eyes open spontaneous	4
	Eyes opening to verbal command	3
	Eyes opening to pain	2
	No eye opening	1
2.	**Motor Response**	
	Obey commands	6
	Localize pain	5
	Withdrawal from pain	4
	Stereotyped flexion to pain	3
	Stereotyped extension to pain	2
	No motor response	1
3.	**Best Verbal Response**	
	Oriented	5
	Confused	4
	Inappropriate words	3
	Incomprehensible sounds	2
	No verbal response	1
4.	**Reaction of pupil to light**	
	Normal	5
	The pupil is slow to respond to light	4
	Pupil reflex is different on both sides	3
	Bilateral pupils are unequal in size	2
	The pupil does not reflect light	1
5.	**Brainstem reflexes**	
	The brainstem reflexes are all present	5
	Eyelash reflexes disappear	4
	Corneal reflexes disappear	3
	Cerebral, ocular and vestibular reflexes disappeare	2
	All these reflections disappeare	1
6.	**Convulsions**	
	No convulsions	5
	Localized convulsions	4
	Paroxysmal convulsions	3
	Continuous convulsions	2
	Relaxed state	1
7.	**Spontaneous respiration**	
	Normal	5
	Periodic breathing	4
	Hyperventilation of the central system	3
	Irregular/hypopnea	2
	Apnoea	1

Table 2
Baseline characteristics and clinical data of the enrolled patients before treatment.

Variable	Fever group	NT group	MH group	*P*-Value
Number	78	113	86	
Female/male	41/37	58/55	44/42	$P = 0.976$
Age (y)	48.3 ± 6.9	46.3 ± 7.3	48.2 ± 7.9	$P = 0.110$
BMI (kg/m^2)	21.5 ± 3.7	22.1 ± 3.9	22.6 ± 3.2	$P = 0.116$
Education level (y)	10.1 ± 3.9	11.2 ± 3.9	10.2 ± 4.1	$P = 0.080$
Work type (Me/Ma)	38/40	54/59	41/45	$P > 0.95$
CO exposure time (h)	5.9 ± 2.7	5.7 ± 2.6	6.0 ± 3.1	$P = 0.619$
Pre-hospital delay(h)	10.1 ± 5.9	11.2 ± 6.8	9.9 ± 7.4	$P = 0.331$
Coma time (h)	18.5 ± 2.8	17.6 ± 3.1	17.8 ± 2.9	$P = 0.573$
Mechanical ventilation (n)	1	2	1	$P = 0.325$
Glucose(mmol/l)	7.5 ± 2.0	7.4 ± 3.1	7.1 ± 2.7	$P = 0.598$
SpO_2(%)	88.2 ± 5.2	90.3 ± 5.6	87.3 ± 4.5	$P < 0.001$
COHb level (%)	28.7 ± 8.4	28.9 ± 9.6	29.4 ± 8.9	$P = 0.899$
G-P scores	8.84 ± 2.05	9.32 ± 2.42	9.29 ± 2.56	$P = 0.336$
APACHE II	24.44 ± 3.07	23.91 ± 2.13	23.63 ± 1.90	$P = 0.089$
HDS scores	6.32 ± 2.28	6.71 ± 2.66	6.59 ± 2.44	$P = 0.569$
MMSE scores	11.21 ± 2.39	11.68 ± 2.79	11.69 ± 2.30	$P = 0.419$
BIS values	53.77 ± 8.73	55.29 ± 8.72	54.19 ± 5.93	$P = 0.392$
NSE index	34.19 ± 8.35	32.47 ± 9.05	33.71 ± 9.16	$P = 0.379$

BMI: body mass index; COHb: carboxyhemoglobin; G-P scores: Glasgow-Pittsburgh coma scale.

As shown in Table 3, there was no significant difference among the three groups in previous chronic underlying diseases ($P > 0.05$).

3.2. Comparison of disease severity among the three groups before and after treatment

Before treatment, it lacked significant difference in the comparasion of the G-P scores and APACHE II scores among the three groups ($P > 0.05$). However, the G-P scores both in the NT and MH groups were increased, and the APACHE II scores were decreased at 1 day, 3 days and 7 days after treatment as compared with the fever group, and there were significant differences ($P < 0.05$, Fig. 4). The therapeutic effect in the MH group was the best among the three groups, suggesting that early mild hypothermia treatment may be beneficial to the recovery of patients with severe CO poisoning.

3.3. Changes of neurobehavioral scores in each group before and after treatment

As shown in Fig. 5, the HDS score and MMSE score in the NT group and the MH group were slightly higher than that in the fever group before treatment, but no significant difference was found among the three groups ($P > 0.05$), suggesting that patients with CO poisoning have neurobehavioral dysfunction. Compared with the fever group, the HDS score and MMSE score in the NT group and the MH group were increased at 7 days, 1 month and 6 months after treatment with significant differences ($P < 0.05$). The therapeutic effect of MH group was better than that of NT group.

3.4. Alternation of BIS value and NSE concentration among the three groups at different time points

Before treatment, the difference of BIS values was not significant among the three groups ($P > 0.05$). Compared with the NT group and the MH group, BIS values in the fever group were decreased on day 1, 3, 7 and month 1, accompanied with significant differences (Fig. 6, $P < 0.05$). BIS values in the NT group were slightly lower than those in the MH group, and the comparison between the two groups showed significant differences at 1 day, 3 days and 7 days after treatment ($P < 0.05$).

Likewise, NSE concentration in serum in the NT group and the MH group were slightly lower than that in the fever group, and there were no significant difference before treatment ($P > 0.05$). However, NSE concentration in the fever group was increased on day 1, 3, 7 and month 1 after therapeutic interventions, accompanied by significant differences compared with the NT group and the MH group ($P < 0.05$). NSE concentration in the NT group was higher than that in the MH group, and the comparison between the two groups was significant differences at 3 days, 7 days and 1 month after treatment ($P < 0.05$, Fig. 6).

3.5. Comparison of O_2UCc levels in three groups at different time points

As shown in Fig. 7, the difference of O_2UCc levels was not significant among the three groups before treatment ($P > 0.05$). On day 1 after the treatment, the O_2UCc level in all three groups fell to different degrees, among which the values in the MH group dropped significantly, followed by the NT group, accompanied by significant differences as compared with the fever group at the same period ($P < 0.05$). The significantly decreased O_2UCc values were observed from day 1 to day 3 after intervention. The therapy improvement in the MH group was very satisfactory than the other two groups ($P < 0.05$), suggesting that mild hypothermia therapy may become a promising treatment strategy. Moreover, at the later stage of treatment (after 1 month), O_2UCc value of patients gradually stabilized and remained at a low level, and the difference was still significant among the three groups ($P < 0.05$).

Table 3
Comparison of chronic underlying diseases among the three groups.

	Fever group (N = 78)	NT group (N = 113)	MH group (N = 86)	*P*-Value
Cardiovascular diseases	8	11	7	0.901
Respiratory diseases	6	9	7	$P > 0.95$
Liver diseases	3	5	4	$P > 0.95$
Kidney diseases	3	3	2	0.816
Other systemic diseases	5	7	6	$P > 0.95$

Note: When N < 10, Fisher exact probability method was used instead of χ^2 test.

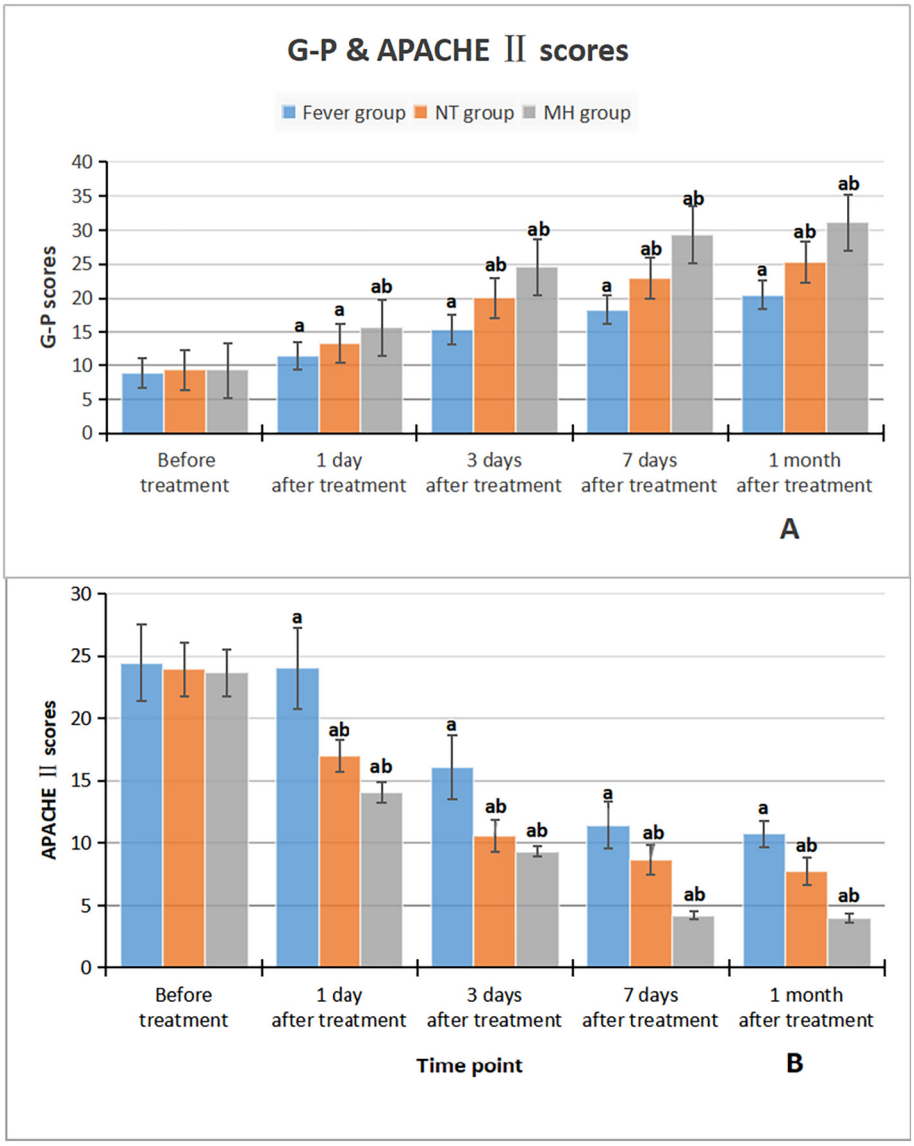

Fig. 4. Comparison of G-P scores and APACHE II scores among three groups.
A: The changes of G-P scores in different time points among three groups.
B: The changes of APACHE II scores in different time points among three groups.
Note: a, Compared with the same group at the day before the treatment, $P < 0.001$; b, Compared with the fever group at the same time points, $P < 0.001$.

3.6. The comparison of the incidence of average duration of coma, delayed encephalopathy and nervous system injury among the three groups after treatment

Coma is an important index to measure the severity of acute CO poisoning. When GCS score is <8, it is recognized as coma in clinical significance in the present study. Nervous system injury refers to the clinical symptoms of the nervous system other than coma caused by acute CO poisoning, such as cognitive disability, dyskinesia, sensory disorders, vegetative impairment, visual dysfunction and so on. The average duration of coma in the fever group was significantly longer than that in the NT group and the MH group ($P < 0.001$, Table 4). The incidence of DEACMP and nervous system injury in the fever group was significantly higher than that in the other two groups ($P < 0.05$). The univariate logistic regression analysis showed that fever (body temperature above 38 °C at the first 3 days after CO poisoning) was a risk factor for DEACMP and nervous system injury compared with the NT group. The OR values were 2.072 and 3.033, and mild hypothermia was the protective factor of DEACMP and nervous system injury compared with the NT group, and the OR values were 0.401 and 0.424, respectively.

Therefore, we put the average duration of coma, groups and other factors into the multivariate logistic regression analysis. We found that the average duration of coma was the risk factor of DEACMP and nervous system injury, and the OR values were 2.513 and 1.259, respectively.

The survival curve showed that the occurrence time of DEACMP and nervous system injury in the MH group was later than that in the other two groups (Fig. 8), and no midway deaths occurred in all three groups at 6 months follow-up.

3.7. Diagnostic value of G-P score, BIS value and NSE concentration in the occurrence of DEACMP

The area under ROC curve (AUC) of G-P score was 0.775. Cut-off point for DEACMP (positive prediction) was as follows: when the G-P score was ≦12.41, the sensitivity was 79.3% and the specificity was 67.6%, respectively (Fig. 9A). The area under ROC curve of BIS value

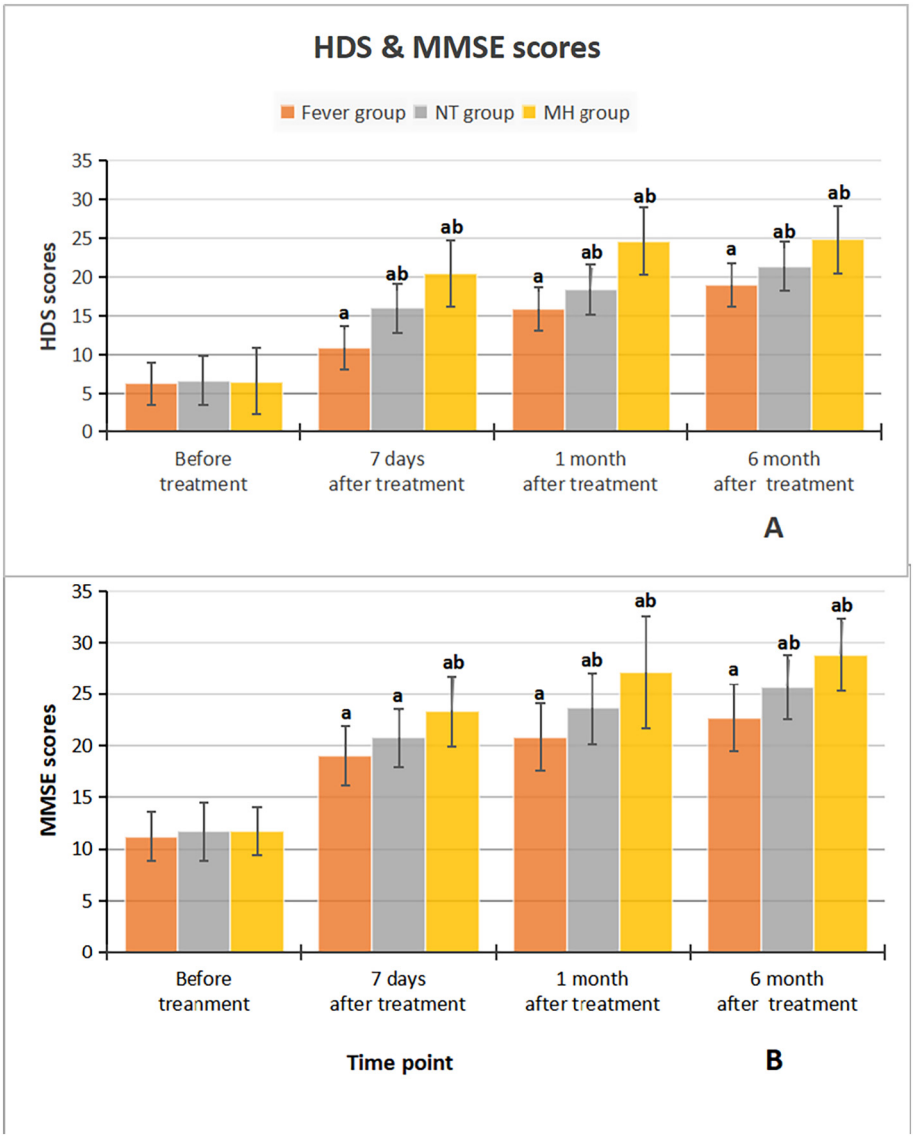

Fig. 5. Comparison of HDS and MMSE scores among three groups.
A: The changes of HDS scores in different time points among three groups.
B: The changes of MMSE scores in different time points among three groups.
Note: a, Compared with the same group at the day before the treatment, $P < 0.001$; b, Compared with the fever group at the same time points, $P < 0.001$.

was 0.876. Cut-off point for DEACMP (positive prediction) was as follows: as the BIS value was ≦ 52.17, the sensitivity was 77.6%, and the specificity was 89.5% (Fig. 9B). The area under the ROC curve of NSE concentration was 0.878. Cut-off point for DEACMP (positive prediction) was as follows: NSE concentration was ≧35.20 ng/mL, the sensitivity was 79.3%, and the specificity was 88.6% (Fig. 9C).

4. Discussion

The nervous system injury caused by CO poisoning is a complex pathological process. Clinically, the therapeutic goal of acute severe CO poisoning is not only to improve brain tissue hypoxia and increase blood oxygen content, but also to actively protect the structure and function of brain tissue, timely eliminate excessive oxygen free radicals [10,11], balance the active state of the patient's antioxidant/oxidative system [12] and improve the immune state of the body.

Clinically, acute brain edema and brain injury after CO poisoning often develop to the peak within 24–48 h and last for 2–3 days. Mild hypothermia treatment should cover the peak period of brain edema and brain injury (3–5 days) in accordance with the Chinese expert consensus for mild hypothermia brain protection [13]. Some literature methods and a recent meta-analysis showed improved outcome when hypothermia was continued for between 48 h and 5 days. The longest time lasted 5–7 days, and the patient could stop after passing through the dangerous period [14-18]. Thus, a total of 5 days was selected as the target duration of hypothermia in our study.

Mild hypothermia has a broad protective effect on various brain tissue injuries, which can reduce oxygen consumption, maintain cell metabolism [19], stabilize the blood-brain barrier, reduce brain edema [20], inhibit excitatory amino acid and free radical damage, and inhibit the activation of inflammatory factors and aggregation in the central nervous system [21]. Clinical studies have confirmed that mild hypothermia treatment is safe and effective for patients with coma and cerebral ischemia after cardiopulmonary resuscitation [22,23]. In current clinical studies, we found that the incidence of DEACMP and the incidence of nervous system injury in the mild hypothermia group were significantly lower than that in the NT group and the fever group,

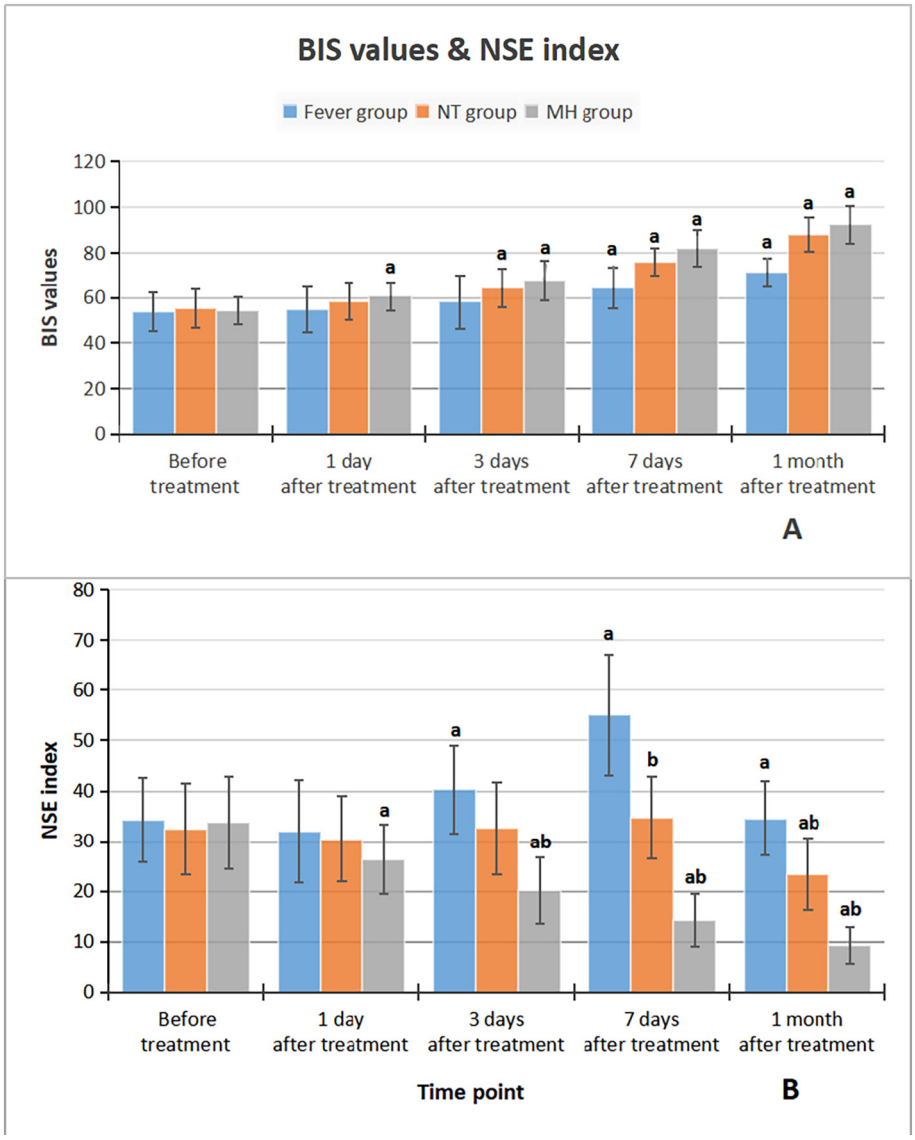

Fig. 6. Comparison of BIS values and NSE concentration index among three groups.
A: The changes of BIS values in different time points among three groups.
B: The changes of NSE concentration index in different time points among three groups.
Note: a, Compared with the same group at the day before the treatment, $P < 0.006$; b, Compared with the fever group at the same time points, $P < 0.006$.

suggesting that mild hypothermia can improve the prognosis of patients with severe CO poisoning.

G-P score and APACHE IIscore are the most commonly used tools to evaluate the degree of coma [13]. In this study, we found that compared with the fever group, G-P score was increased in both NT and MH groups, while APACHE II score was decreased, and this therapeutic effect was significant in the MH group, indicating that mild hypothermia therapy can alleviate the patient's consciousness disorder and improve the patient's nervous system function and other physiological functions. As we know, the coma time of patients with CO poisoning is mainly related to some factors, such as the severity of poisoning, the time of patients staying in the carbon monoxide environment, drug intervention, and whether the follow-up treatment is timely and effective. There was no significant difference in some factors above among the three groups before treatment. In the present study, we found that the average duration of coma was the risk factor of DEACMP and nervous system injury, and the OR values were 2.513 and 1.259, respectively, that is to say, the longer average duration of coma, the more chance of patients with DEACMP and nervous system injury.

HDS and MMSE rating scales are the most widely used screening tools for Alzheimer's disease in the world. The MMSE scale reflects the mental state and cognitive impairment of the subjects comprehensively, accurately and quickly [24]. HDS is an early screening tool for dementia, easy to use and less influenced by education [25]. In the present study, we found that HDS scores and MMSE scores were significantly higher in the NT and MH groups at 7 days, 1 month and 6 months of treatment compared with the fever group, indicating that mild hypothermia treatment could significantly improve the cognitive function and protect the nervous system of patients with severe CO poisoning especially in the later stages of treatment (7 days to 6 months).

BIS index is to measure the linear components (frequency and power) of electroencephalogram (EEG), and analyze the nonlinear relationship (phase and harmonic) between component waves [26]. Various EEG signals representing different sedation levels are selected, standardized and digitized, and finally transformed into a simple quantitative index. It is often used to monitor the depth of the patient's anesthesia. This index can quickly and accurately reflect the status of cerebral cortex function, including changes in brain cell metabolism

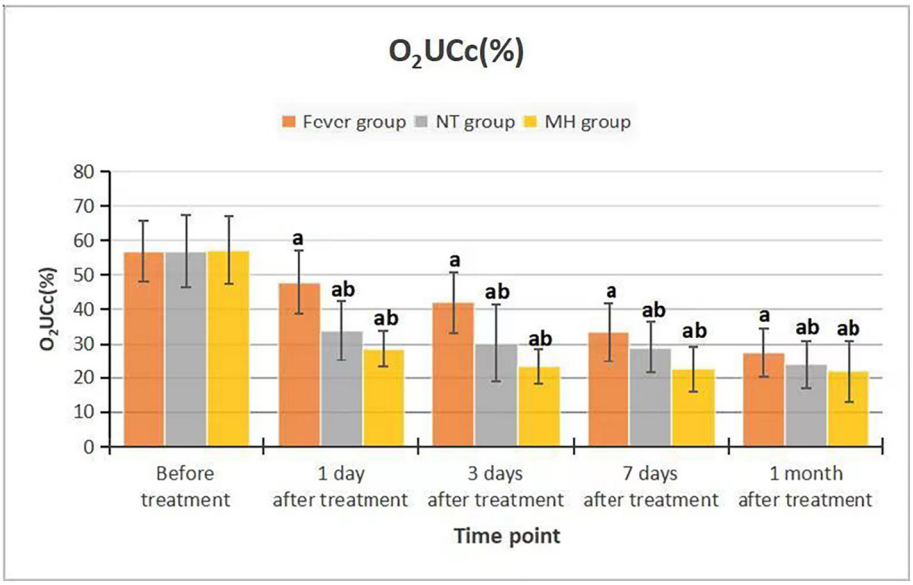

Fig. 7. Comparison of $O_2UCc\%$ in different time periods among three groups.
Note: a, Compared with the same group at the day before the treatment, $P < 0.001$; b, Compared with the fever group at the same time points, $P < 0.001$.

and brain injury caused by hypoperfusion, ischemia and hypoxia. Currently, a large number of studies have shown that BIS index can be used as an effective indicator for early prognosis assessment of patients after cerebral resuscitation [27]. In this study, BIS index in patients with fever decreased significantly on day 1, 3, 7, and month 1 compared with those in the NT and the MH groups. BIS values of patients in the NT group were slightly lower than those in the MH group at 1 day, 3 days and 7 days of treatment, indicating that early mild hypothermia therapy is helpful to reduce brain damage and effectively improve the functional state of cerebral cortex.

Serum NSE, an enolase involved in glycolytic pathway, is mainly found in the cytoplasm of neurons in the central nervous system, and is an objective indicator for the evaluation of neuronal injury [28]. The level of NSE is very low in normal human fluids. When neurons are damaged or necrotized due to ischemia, hypoxia, or toxic injury, NSE spills from the cells and enters the cerebrospinal fluid and blood. Studies have found that NSE is positively correlated with the severity of cognitive dysfunction [29], and can be used to evaluate the degree of cognitive dysfunction in DEACMP patients. In this study, NSE concentration in the fever group was significantly increased on day 1, 3, 7 and month 1 compared with that in the NT and the MH groups ($P < 0.05$). NSE concentration in the NT was higher than that in the MH group at 3, 7 days and 1 month of treatment. These data provide a strong evidence that NSE is closely related to the incidence of the onset encephalopathy [30]. Early mild hypothermia treatment can reduce NSE concentration in serum, inhibit neuron injury, and play a neuroprotection in cerebral tissue.

Cerebral oxygen utilization rate (O_2UCc) can reflect the relationship between cerebral blood perfusion and cerebral metabolism, and correctly reflect the cerebral blood circulation state. A retrospective study found that O_2UCc was correlated with the occurrence of delayed encephalopathy, and the dynamic change of O_2UCc was of reference value for the early identification of DEACMP, and O_2UCc could be used as an important reference index to predict DEACMP. In our study, we found that compared with the NT and the MH groups, BIS values in the fever group were significantly decreased on day 1, 3, 7 and month 1, indicating that fever can aggravate the imbalance between cerebral blood perfusion and cerebral metabolism after CO poisoning. Early reasonable MH treatment can effectively improve cerebral blood perfusion and metabolism in patients with acute severe CO poisoning. At the later stage of treatment (1 month), the O_2UCc value of patients gradually stabilized and remained at a low level. Thus, the early intervention of mild hypothermia has a significant effect on the rehabilitation of brain function after CO poisoning.

It is particularly important to monitor the brain function and determine the degree of damage in patients with severe CO poisoning in early stage. In this study, we found that the changes of G-P score, BIS index, NSE concentration have high sensitivity and specificity for the diagnosis of delayed encephalopathy, and the detection of the three indicators above can make up for the deficiency and limitations of clinical manifestations, imaging features and nerve electrophysiology examination, simultaneously. Moreover, the monitoring process is convenient, repeatable, and clinically significant to the early evaluation of cerebral nerve injury, the assessment of curative effect and the prognosis in patients with acute severe CO poisoning.

Recently, there are many studies on mild hypothermia for severe patients. For example, hypothermia is used to treat cardiac arrest patients, but the conclusion was different from what was expected.

Table 4
Comparison of average duration of coma, incidence of delayed encephalopathy and nervous system injury among the three groups after treatment (%).

Group	N	Mean duration of coma (h)	Incidence of delayed encephalopathy [n(%)]	Incidence of nervous system injury [n(%)]
Fever group	78	16.59 ± 6.27	27(34.61%)	33(42.31%)
NT group	113	10.36 ± 5.15	23(20.35%)	22(19.47%)
MH group	86	8.77 ± 3.25	8(9.30%)	8(9.30%)
OR value			1.640	2.748
P-value		$P < 0.001$	$P < 0.001$	$P < 0.001$

Note: Fever (body temperature above 38 °C at the first 3 days after CO poisoning) was the risk factor of delayed encephalopathy and nervous system injury, and the odds ratios (OR) value were 1.640 and 2.748, respectively.

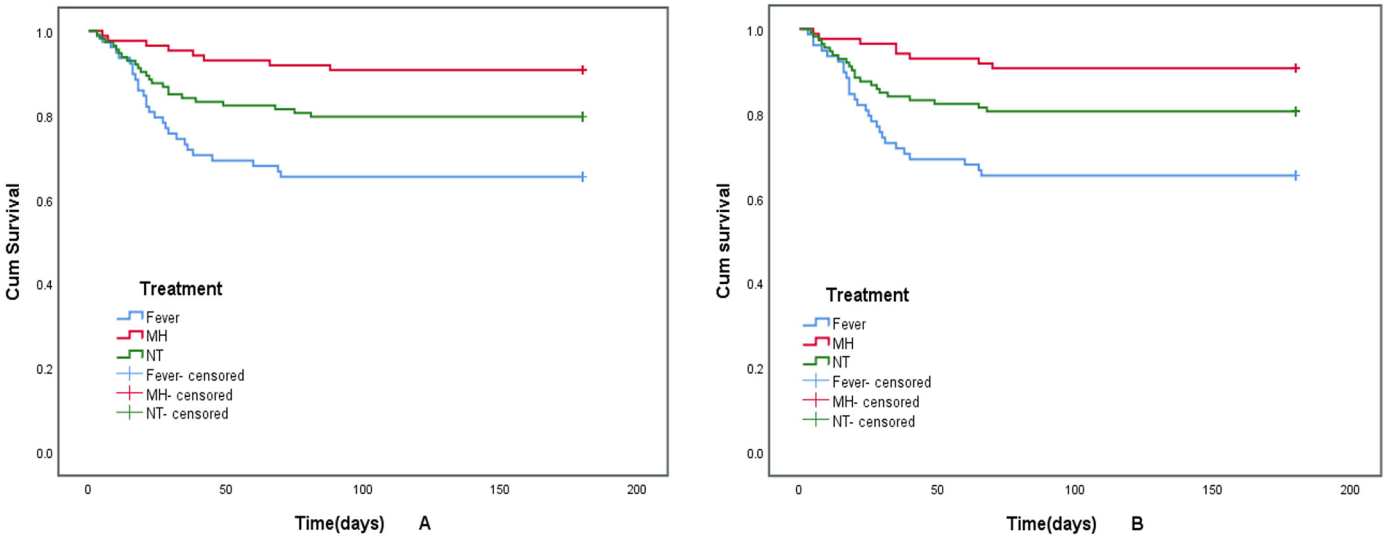

Fig. 8. Comparison of survival curve of patients among three groups.
A, Kaplan-Meier survival curve of patients with DEACMP; B, Kaplan-Meier survival curve of patients with nerve injury.

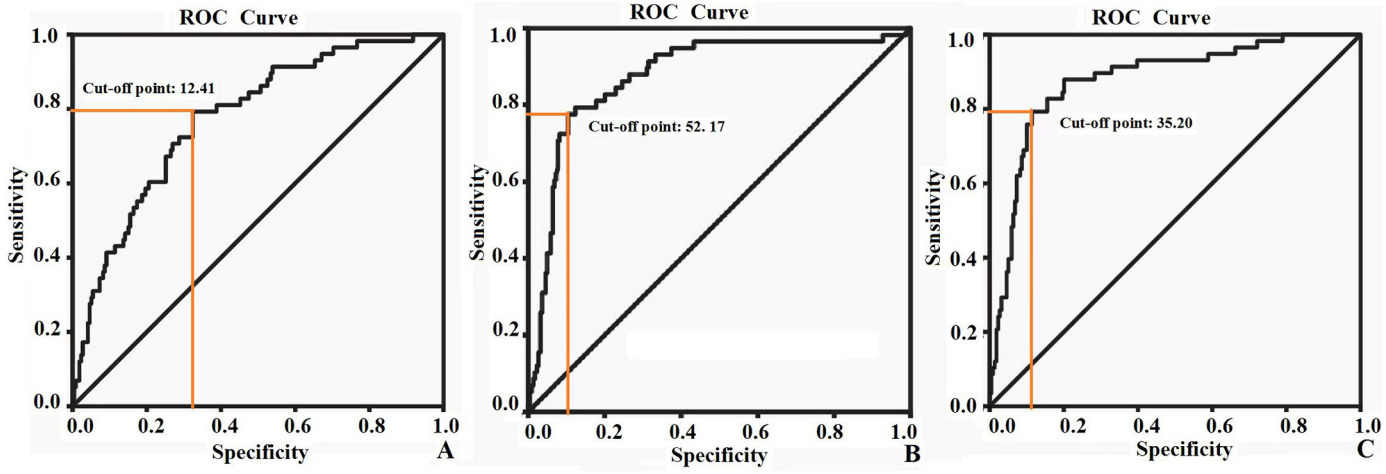

Fig. 9. Diagnostic value of G-P score (A), BIS value (B) and NSE concentration monitoring (C) for the occurrence of delayed encephalopathy.

The result shows that in patients with coma after out-of-hospital cardiac arrest, targeted hypothermia did not lead to a lower incidence of death by 6 months than targeted normothermia [31]. However, our study showed that early mild hypothermia treatment could significantly reduce the severity of brain injury after CO poisoning. It may be related to the difference of target organs, induction methods and durations, sample size and disease type of enrolled patients in the treatment of mild hypothermia.

5. Limitations of the study

The present study still has limitations. On the one hand, the non-randomized design and small sample size limited definite conclusions on the clinical benefit of mild hypothermia for the treatment of CO poisoning. On the other hand, although there were clear inclusion and exclusion criteria, the delay time before admission of patients was different (within 24 h), which might affect the final experimental results. Thus, the specific mechanism of mild hypothermia in the treatment of brain injury caused by CO poisoning needs to be further studied in the future.

6. Conclusions

Mild hypothermia therapy can decrease the occurrence of delayed encephalopathy and improve the prognosis of patients with CO poisoning. However, the sample size of this study is small, and further exploration is still needed in the follow-up.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the institutional review board of Qingdao University in Clinicaltrials.qd.edu (QDCT00506747). All medical ethical approval was granted from Qingdao University Research Ethics Committee (Clinical trial registry and ethical approval number: QD81571283). The patients/participants provided their written informed consent to participate in this study.

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Conflicts of interest

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The data involved in this paper does not include the data, such as Sequence/expression data, protein/molecule characterizations, annotations, and taxonomy data. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. Besides, all experiment processes have been adhered to the standard biosecurity and institutional safety procedures, including the collection, treatment and destruction of patients' samples.

All authors have read and approved the manuscript, and ensure that this is the case.

All authors declare that they have no conflicts of interest.

CRediT authorship contribution statement

Jing-Jing Zhang: Writing – original draft. **Wei-Kang Bi:** Writing – original draft, Conceptualization. **Yong-Mei Cheng:** Methodology. **Ao-Chun Yue:** Methodology, Data curation. **Hui-Ping Song:** Formal analysis, Data curation. **Xu-Dong Zhou:** Visualization, Resources, Project administration. **Ming-Jun Bi:** Software, Investigation, Funding acquisition, Formal analysis. **Wei Han:** Writing – review & editing, Supervision, Formal analysis. **Qin Li:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

None.

References

[1] Yu H, Wu Z, Wang X, Gao C, Liu R, Kang F, et al. Protective effects of combined treatment with mild hypothermia and edaravone against cerebral ischemia/reperfusion injury via oxidative stress and Nrf2 pathway regulation. Int J Oncol. 2020;57(2):500–8. https://doi.org/10.3892/ijo.2020.5077.

[2] Tu Y, Guo C, Song F, Huo Y, Geng Y, Guo M, et al. Mild hypothermia alleviates diabetes aggravated cerebral ischemic injury via activating autophagy and inhibiting pyroptosis. Brain Res Bull. 2019;150:1–12. https://doi.org/10.1016/j.brainresbull.2019.05.003.

[3] Xu SY, Hu YF, Li WP, Wu YM, Ji Z, Wang SN, et al. Intermittent hypothermia is neuroprotective in an in vitro model of ischemic stroke. Int J Biol Sci. 2014;10(8):873–81. https://doi.org/10.7150/ijbs.8868.

[4] Oh BJ, Im YG, Park E, Min YG, Choi SC. Treatment of acute carbon monoxide poisoning with induced hypothermia. Clin Exp Emerg Med. 2016;3(2):100–4. https://doi.org/10.15441/ceem.15.034.

[5] Feldman J, Renda N, Markovitz GH, Chin W, Sprau SE. Treatment of carbon monoxide poisoning with hyperbaric oxygen and therapeutic hypothermia. Undersea Hyperb Med. 2013;40(1):71–9.

[6] Wolf SJ, Maloney GE, Shih RD, Shy BD, Brown MD. Clinical policy: critical issues in the evaluation and management of adult patients presenting to the emergency department with acute carbon monoxide poisoning. Ann Emerg Med. 2017;69(1):98–107.e6. https://doi.org/10.1016/j.annemergmed.2016.11.003.

[7] Yan L, Liang T, Cheng O. Hyperbaric oxygen therapy in China. Med Gas Res. 2015;5:3. https://doi.org/10.1186/s13618-015-0024-4.

[8] Akavipat P, Thinkhamrop J, Thinkhamrop B, Sriraj W. Acute physiology and chronic health evaluation (APACHE)IIscore-the clinical predictor in neurosurgical intensive care nit. Acta Clin Croat. 2019;58(1):50–6. https://doi.org/10.20471/acc.2019.58.01.07.

[9] Schuller PJ, Newell S, Strickland PA, Barry JJ. Response of bispectral index to neuromuscular block in awake volunteers. Br J Anaesth. 2015;115(Suppl. 1):i95–i103. https://doi.org/10.1093/bja/aev072.

[10] Xu XM, Luo H, Rong BB, Zheng XM, Wang FT, Zhang SJ, et al. Management of delayed encephalopathy after CO poisoning: an evidence-based narrative review. Medicine (Baltimore). 2019;98(49):e18199. https://doi.org/10.1097/MD.0000000000018199.

[11] Bi M, Li Q, Guo D, Ding X, Bi W, Zhang Y, et al. Sulphoraphane improves neuronal mitochondrial function in brain tissue in acute carbon monoxide poisoning rats. Basic Clin Pharmacol Toxicol. 2017;120(6):541–9. https://doi.org/10.1111/bcpt.12728.

[12] Li Q, Bi MJ, Bi WK, Kang H, Yan le J, Guo YL. Edaravone attenuates brain damage in rats after acute CO poisoning through inhibiting apoptosis and oxidative stress. Environ Toxicol. 2016;31(3):372–9. https://doi.org/10.1002/tox.22052.

[13] Cerebral protection in cardiac intensive care group neural regeneration and repair committee Chinese research hospital association, neural intensive nursing and rehabilitation group neural regeneration and repair committee Chinese research hospital association. Chinese consensus for mild hypothermia brain protection. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue. 2020;32(4):385–91. Chinese. https://doi.org/10.3760/cma.j.cn121430-20200117-00137.

[14] Kim F, Nichol G, Maynard C, Hallstrom A, Kudenchuk PJ, Rea T, et al. Effect of prehospital induction of mild hypothermia on survival and neurological status among adults with cardiac arrest: a randomized clinical trial. JAMA. 2014;311(1):45–52. https://doi.org/10.1001/jama.2013.282173.

[15] Shin J, Kim J, Song K, Kwak Y. Core temperature measurement in therapeutic hypothermia according to different phases: comparison of bladder, rectal, and tympanic versus pulmonary artery methods [J]. Resuscitation. 2013;84(6):810–7. https://doi.org/10.1016/j.resuscitation.2012.12.023.

[16] Dididze M, Green BA, Dietrich WD, Vanni S, Wang MY, Levi AD. Systemic hypothermia in acute cervical spinal cord injury: a case-controlled study. Spinal Cord. 2013;51(5):395–400. https://doi.org/10.1038/sc.2012.161.

[17] Andrews PJ, Sinclair HL, Battison CG, Polderman KH, Citerio G, Mascia L, et al. European society of intensive care medicine study of therapeutic hypothermia (32-35 °C) for intracranial pressure reduction after traumatic brain injury (the Eurotherm3235Trial). Trials. 2011;12:8. https://doi.org/10.1186/1745-6215-12-8.

[18] Clifton GL. A review of clinical trials of hypothermia treatment for severe traumatic brain injury. Ther Hypothermia Temp Manag. 2011;1(3):143–9. https://doi.org/10.1089/ther.2011.0009.

[19] Burns JD, Fisher JL, Cervantes-Arslanian AM. Recent advances in the acute management of intracerebral hemorrhage. Neurol Clin. 2017;35(4):737–49. https://doi.org/10.1016/j.nec.2017.11.005.

[20] Kim H, Edwards NJ, Choi HA, Chang TR, Jo KW, Lee K. Treatment strategies to attenuate perihematomal edema in patients with intracerebral hemorrhage. World Neurosurg. 2016;94:32–41. https://doi.org/10.1016/j.wneu.2016.06.093.

[21] Wu D, Shi J, Elmadhoun O, Duan Y, An H, Zhang J, et al. Dihydrocapsaicin (DHC) enhances the hypothermia-induced neuroprotection following ischemic stroke via PI3K/Akt regulation in rat. Brain Res. 2017;1671:18–25. https://doi.org/10.1016/j.brainres.2017.06.029.

[22] Wiklund L, Patnaik R, Sharma A, Miclescu A, Sharma HS. Cerebral tissue oxidative ischemia-reperfusion injury in connection with experimental cardiac arrest and cardiopulmonary resuscitation: effect of mild hypothermia and methylene blue. Mol Neurobiol. 2018;55(1):115–21. https://doi.org/10.1007/s12035-017-0723-z.

[23] Li L, Yang R, Li P, Lu H, Hao J, Li L, et al. Combination treatment with methylene blue and hypothermia in global cerebral ischemia. Mol Neurobiol. 2018;55(3):2042–55. https://doi.org/10.1007/s12035-017-0470-1.

[24] Hu C, Wang L, Zhao X, Zhu B, Tian M, Qin H. Investigation of risk factors for the conversion of mild cognitive impairment to dementia. Int J Neurosci. 2020:1–14. https://doi.org/10.1080/00207454.2020.1782905.

[25] Rosli R, Tan MP, Gray WK, Subramanian P, Chin AV. Cognitive assessment tools in Asia: a systematic review. Int Psychogeriatr. 2016;28(02):189–210. https://doi.org/10.1017/S1041610215001635.

[26] Pappal RD, Roberts BW, Winkler W, Yaegar LH, Stephens RJ, Fuller BM. Awareness and bispectral index (BIS) monitoring in mechanically ventilated patients in the emergency department and intensive care unit: a systematic review protocol. BMJ Open. 2020;10(3):e034673. https://doi.org/10.1136/bmjopen-2019-034673.

[27] Eveson L, Vizcaychipi M, Patil S. Role of bispectral index monitoring and burst suppression in prognostication following out-of-hospital cardiac arrest: a systematic review protocol. Syst Rev. 2017;6(1):191. https://doi.org/10.1186/s13643-017-0584-6.

[28] Kamenik M, Möller Petrun A. Bispectral index-guided induction of general anaesthesia. Br J Anaesth. 2014;112(1):169. https://doi.org/10.1093/bja/aet445.

[29] Stammet P. Blood biomarkers of hypoxic-ischemic brain injury after cardiac arrest. Semin Neurol. 2017;37(01):75–80. https://doi.org/10.1055/s-0036-1593858.

[30] Cha YS, Kim H, Do HH, Kim HI, Kim OH, Cha KC, et al. Serum neuron-specific enolase as an early predictor of delayed neuropsychiatric sequelae in patients with acute carbon monoxide poisoning. Hum Exp Toxicol. 2018;37(3):240–6. https://doi.org/10.1177/0960327117698544.

[31] Dankiewicz J, Cronberg T, Lilja G, Jakobsen JC, Bělohlávek J, Callaway C, et al. Targeted hypothermia versus targeted Normothermia after out-of-hospital cardiac arrest (TTM2): a randomized clinical trial-rationale and design. Am Heart J. 2019;217:23–31. https://doi.org/10.1016/j.ahj.2019.06.012.