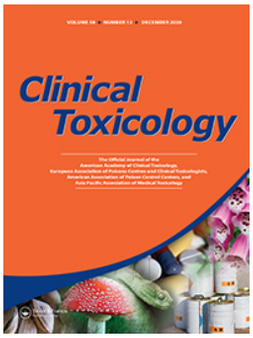

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CLINICAL RESEARCH



# Serum netrin-1 levels at presentation and delayed neurological sequelae in unintentional carbon monoxide poisoning

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**ABSTRACT**

**Objectives:** The early identification of patients with a high risk of developing delayed neurological sequelae (DNS) can improve the quality of care in carbon monoxide (CO) poisoning cases. The aim of this study is to investigate whether the serum netrin-1 levels measured at presentation to the emergency department (ED) predicted the development of DNS after acute CO intoxication.

**Methods:** This prospective observational study was conducted between 1 August 2018 and 31 July 2019 in a single tertiary hospital. The patients with acute CO intoxication and serum netrin-1 levels measured at the time of ED presentation were included in the study. All patients were followed up for six weeks regarding the development of DNS. The patients were divided into two groups, including those who developed DNS (DNS group) and those who did not (non-DNS group).

**Results:** A total of 183 patients were included in the study, and 54 (29.5%) developed DNS. The median serum netrin-1 level at ED presentation was significantly lower in the DNS group (391.5 pg/mL [263.0–550.5]) than in the non-DNS group (626.0 pg/mL [505.9–755.6]) ($p < .001$). Multivariate analysis revealed that a low serum netrin-1 level (adjusted odds ratio [AOR]: 8.02, 95% CI: 2.45–26.20), low Glasgow coma scale (GCS) score at ED presentation (AOR: 0.81, 95% CI: 0.68–0.97), long CO exposure time (AOR: 1.96, 95% CI: 1.49–2.56), and the presence of acute brain lesions (AOR: 8.24, 95% CI: 2.37–28.58) on diffusion-weighted imaging were independent predictors of DNS. Serum netrin-1 levels less than 432 pg/mL predicted the development of DNS with a sensitivity of 68.5% (95% CI: 54.4%-80.5%) and a specificity of 86.0% (95% CI: 78.8%-91.5%).

**Conclusions:** Low serum netrin-1 levels were significantly associated with the development of DNS. Therefore, serum netrin-1 at ED presentation can help identify patients at risk of developing DNS following discharge.

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**KEYWORDS**
Netrin-1; carbon monoxide; poisoning; delayed neurological sequelae

## Introduction

Carbon monoxide (CO) poisoning is one of the leading causes of morbidity and mortality worldwide [1,2]. It is estimated that in the United States alone, approximately 100,000 people are affected by CO poisoning annually [2]. While deaths due to CO poisoning have been continuously decreasing for the last two decades, the actual problem today is the development of neurological sequelae in surviving patients after acute treatment [3–5]. Neurological sequelae may occur in the acute phase of CO poisoning. However, delayed neurological sequelae (DNS) develop in some cases after the achievement of full recovery in the acute phase [1,4,6]. DNS usually occurs within six weeks after the disappearance of acute poisoning symptoms [7,8]. Therefore, it is very important for the follow-up decision and treatment planning in clinical practice to predict the patients that will develop DNS. However, to date, no reliable method has been presented to determine the likelihood of DNS development after CO poisoning [4,9].

The use of biochemical markers to identify neuronal damage in the early stage of CO poisoning can provide objective data that can help predict the development of DNS. Netrin-1 is a protein that regulates angiogenesis and the migration of neuron cells to damaged regions of the central nervous system [10,11]. Netrin-1 is also involved in the regulation of the blood-brain barrier, has anti-inflammatory effects, and has been reported to inhibit the apoptosis of neuron cells [12–14]. This protein and its receptor have been reported to be associated with psychiatric diseases, such as depression and schizophrenia [15]. Serum netrin-1 levels have been extensively investigated as a predictive marker of neurological sequelae following ischemic stroke, hemorrhagic stroke, and subarachnoid hemorrhage [16–18]. However, to date, no studies have been conducted on the utility of serum netrin-1 in the prediction of DNS in patients with acute CO poisoning.

The primary aim of this study was to evaluate whether serum netrin-1 levels could be used as a biochemical marker to predict DNS in acute unintentional CO poisoning. Our

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Supplemental data for this article can be accessed here.

secondary aim was to identify the clinical factors that could help predict DNS.

## Materials and methods

### Study design

This prospective observational study was performed in the ED of a tertiary care hospital affiliated with a university. The average annual number of patients presenting to this ED is 300,000. The study was approved by the institutional review board of the hospital (approval no: 2018-16/138). Prior to participating in the study, the patients or their family members provided written consent.

### Study setting and population

The study included all adult (age $\geq$ 18) patients who consecutively presented to the ED with acute unintentional CO poisoning between 1 August 2018 and 31 July 2019 and whose serum netrin-1 level was measured at presentation. Acute unintentional CO poisoning was defined as the presence of a history of accidental exposure to CO and carboxyhemoglobin (COHb) levels greater than 5% (10% for smokers) at ED presentation. CO poisoning cases due to fire accidents were not included in the study because in such cases, exposure to other gases, such as hydrogen cyanide and hydrogen sulfide may also occur. Intentional CO poisoning cases were not included in the study for two important reasons: First, intentional CO poisoning is typically associated with suicidal purposes, and the patients in this group often take overdose of various drugs in addition to CO that have different toxicities, such as sedative hypnotics and antipsychotics [19]. Second, unlike Far East countries [20,21], the use of CO in suicide attempts in Turkey is very rare. Other exclusion criteria included the following: patients who developed cardiac arrest prior to ED presentation, pregnancy, patients whose serum netrin-1 levels could not be measured, the presence of other conditions that could change the serum netrin-1 level, such as previous stroke or subarachnoid hemorrhage [16–18], refusing to participate in the study, previous history of neurocognitive dysfunction (e.g., Parkinson's disease, dementia, and psychiatric diseases) before the CO poisoning event, acute persistent neurological deficits at the time of discharge, and patients who could not be followed up for DNS after discharge. The patients presenting with acute neurological deficit that fully recovered at discharge were not excluded from the study.

### Study protocol

All patients were given 100% oxygen with a non-rebreather face mask in the ED. The patients that required intubation were endotracheally intubated, and 100% oxygen ($FiO2 = 1.0$) was delivered by a mechanical ventilator. Those with presence of indications for hyperbaric oxygen therapy (HBOT) were treated with HBOT using a monoplace hyperbaric chamber. The total HBOT time was 90 min per session, and the target pressure was 2.8 standard atmospheres. The indications for HBOT included COHb level $>$ 25%, neurological signs, such as altered mental status, syncope, seizures, focal neurological deficit, and signs of myocardial ischemia [22]. If a patient with HBOT indications also required intubation, they were intubated and provided 100% oxygen by a mechanical ventilator instead of HBOT.

Diffusion-weighted magnetic resonance imaging (DW-MRI) was performed for all patients as soon as possible after presentation to the ED to assess the severity of acute cerebral injury. Previous studies have shown that acute brain lesions detected on DW-MRI during the acute phase of carbon monoxide poisoning may predict DNS [9,23]. Acute brain lesions were defined as newly formed, prominent, and bright lesions with high signal intensity on DW-MRI. High signal intensity due to chronic lesions was not accepted as an indication of acute brain lesions [9]. For the DW-MRI examination, a 1.5-T MRI unit (Magnetom Amira; Siemens Healthcare, Erlangen, Germany) was used. The results of DW-MRI were evaluated by a radiologist blinded to the purpose and clinical data of the study.

During their hospital stay, all patients and their accompanists were informed about the signs and symptoms of DNS to raise their awareness of DNS. All patients were called for a routine neurology outpatient follow-up visit six weeks after discharge for the evaluation of DNS development. In addition, if the patients had new neurological symptoms before the end of six weeks, they were advised to immediately refer to the neurology outpatient clinic instead of waiting until the end of this period. The diagnosis of DNS was made by a neurologist through a detailed examination and neuropsychiatric tests, such as the Montreal Cognitive Assessment and verbal Buschke's test. In addition, the severity of DNS was assessed with the modified Rankin Scale score six weeks after poisoning. DNS was defined as any new neurological symptoms or signs, including dysarthria, dysphagia, dyspraxia, cognitive decline, parkinsonism, motor deficits, seizures, psychosis, and mood disorders, which developed within six weeks of discharge from the ED [9,24]. Finally, the patients were divided into two groups based on whether they developed DNS and included the DNS group and the non-DNS group.

### Measures

We prospectively collected demographics data (e.g., age, sex), clinical features, and clinical outcomes. Clinical features included the duration of CO exposure, the source of CO, time from the end of CO exposure to arrival at the ED, time from arrival at the ED to DW-MRI scan, comorbid diseases, smoking status, DW-MRI images, the level of consciousness, initial vital signs, and laboratory results including the serum netrin-1 level at the time of presentation. Consciousness level was evaluated according to the Glasgow coma scale (GCS). Clinical outcomes included the duration of hospital stay, survival status, and development of DNS after discharge.

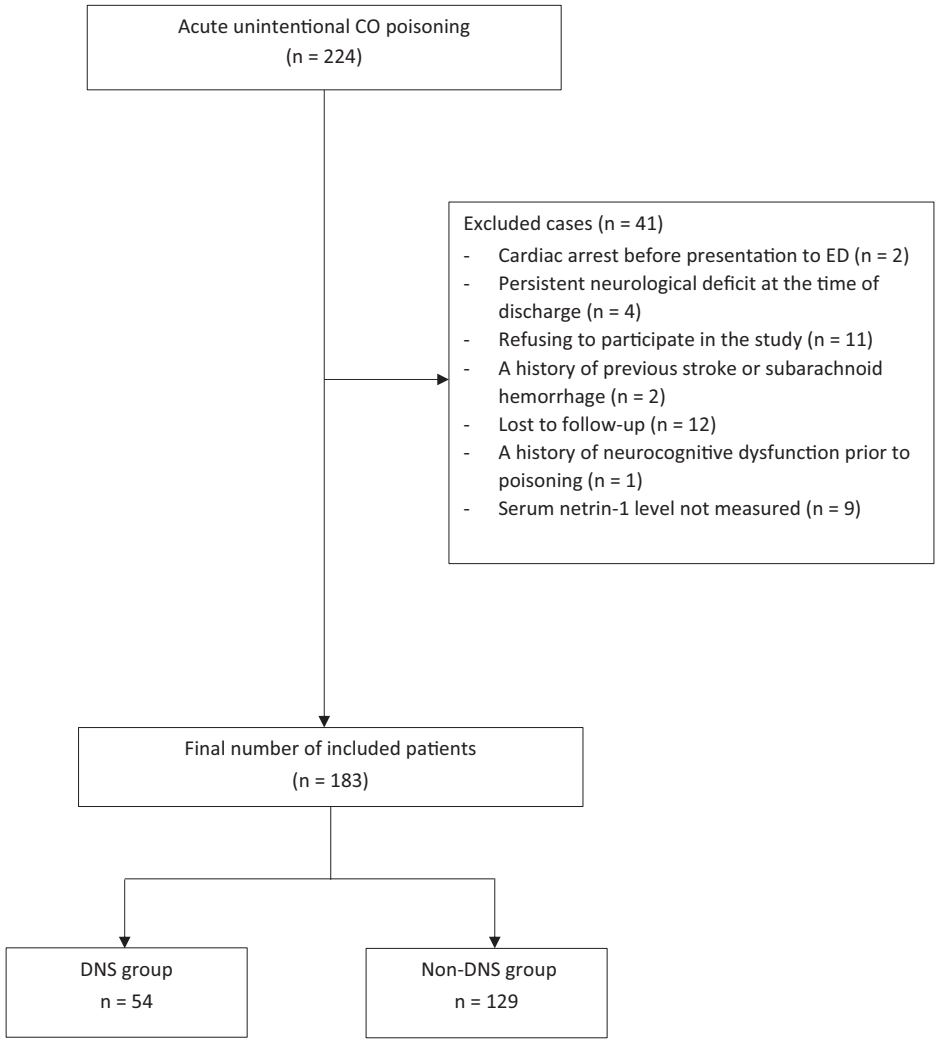


Figure 1. Flow chart of patient selection. DNS: delayed neurological sequelae; CO: carbon monoxide.

### Netrin-1 analysis

Venous blood samples were collected from the patients within the first five minutes of their presentation to the ED. All serum samples were stored at −80 °C according to the manufacturer's instructions until assayed. Netrin-1 concentrations in the serum were measured using a quantitative sandwich ELISA detection kit (Human Netrin-1 ELISA kit, Code Number: CSB-E11899h, Cusabio) according to the protocols provided by the manufacturer. The measurements were read at 450 nm on the microplate reader of a Multiskan FC microplate photometer (ThermoScientific). The results were expressed as pg/mL. The interassay and intraassay variation coefficients were <10.0% and <8.0%, respectively.

### Data analysis

All statistical analyses were performed using SPSS for Windows version 15.0 (SPSS Inc., Chicago, IL, USA). Continous variables were expressed as mean ± standard deviation (SD) and median (interquartile range [IQR]) according to the normality of the distribution. The normality of the variables was determined using the Kolmogorov-Smirnov test. The categorical data were presented as absolute numbers (percentages). Student's *t*-test was used to compare normally distributed continuous variables while Mann-Whitney U test was used for non-normally distributed variables. The Chi-square or Fisher's exact test was used to compare categorical variables. A receiver operating characteristic (ROC) curve was used to determine the optimal cut-off value for the serum netrin-1 level to predict the development of DNS. In addition, the area under the curve (AUC) of the serum netrin-1 level was obtained to predict DNS development. To determine the independent predictors of DNS, clinically significant baseline characteristics and variables that were suggested to be associated with DNS in the literature (such as age, GCS at presentation, creatinine, arterial HCO3-, COHb, DW-MRI results, and length of hospital stay) [6,9,20,25–27] were first evaluated using univariate logistic regression analysis. The variables that were confirmed to be significantly associated with DNS according to univariate analysis ($p < .05$) were further analyzed using multivariate logistic regression analysis based on the backward stepwise method. Hosmer-Lemeshow test was used to test the fittness of the multivariate logistic regression model. The sensitivity and specificity of DW-MRI and serum netrin-1 in predicting DNS were calculated. $p$ value of $< .05$ was considered statistically significant.

## Results

A total of 224 patients with acute unintentional CO poisoning presented to our ED during the study period. Forty-one of these patients were excluded from the study for the following reasons: two developed cardiac arrest prior to ED presentation, four had an acute persistent neurological deficit at the time of discharge, 11 refused to participate in the study, two had a history of previous stroke, one had a history of neurocognitive dysfunction prior to poisoning, serum netrin-1 levels could not be measured in nine patients, and 12 patients were lost to follow-up (Figure 1). As a result, a total of 183 patients were included in this study and were divided into the DNS ($n = 54$, 29.5%) group and the non-DNS ($n = 129$, 70.5%) group. The neurological findings and symptoms of the patients that developed DNS were presented in eTable 1 (Supplementary eTable 1). The median modified Rankin Scale score of the patients with DNS was 2 (2-3).

The baseline clinical characteristics of the patients were summarized in Table 1. The COHb and serum netrin-1 levels at ED presentation were 28.2 ± 9.9% and 578.1 (413.8–722.0) pg/mL, respectively. The serum netrin-1 levels of the patients in the DNS and non-DNS groups are presented in eFigure 1 (Supplementary eFigure 1). There was no significant difference between the DNS and non-DNS groups in terms of age, gender, source of CO, initial vital signs, and COHb levels. The duration of CO exposure in the DNS group was significantly longer than in the non-DNS group (7.3 h vs. 2.5 h, $p < .001$). Compared with the non-DNS group, the initial GCS was significantly lower in the DNS group (15.0 vs 8.0, $p < .001$). The median serum netrin-1 level was significantly lower in the DNS group than in the non-DNS group (391.5 pg/mL vs. 626.0 pg/mL, $p < .001$). The serum netrin-1 levels of the patients who received and did not receive HBOT were 547.3 ± 212.2 and 595.4 ± 221.9 pg/mL, respectively ($p = .148$). From patients who received HBOT; Serum netrin-1 levels of those who developed DNS were significantly lower than those who did not develop DNS (384.6 [241.5–430.0] vs. 636.7 [531.6–738.5] pg/mL, $p < .001$). The DW-MRI examinations revealed acute brain lesions in 72.2% ($n = 39$) of the patients in the DNS group and 13.2% ($n = 17$) of those in the non-DNS group ($p < .001$) (Table 1). No mortality occurred at the hospital or after discharge.

The AUC of serum netrin-1 in the ROC curve for the prediction of DNS development was calculated as 0.802 (95% CI: 0.736–0.857). The optimal cut-off value of serum netrin-1 was 432 pg/mL (Figure 2). In univariate analysis, the following factors were found to be associated with DNS development: hypertension, duration of CO exposure, GCS at presentation, serum netrin-1 level, white blood cell (WBC) count, creatinine, lactate, and presence of an acute brain lesion on DW-MRI. The multivariate logistic regression analysis revealed that longer CO exposure duration (adjusted OR [AOR]: 1.96, 95% CI: 1.49–2.56), lower GCS at presentation (AOR: 0.81, 95% CI: 0.68–0.97), lower serum netrin-1 level (AOR: 8.02, 95% CI: 2.45–26.20), and presence of an acute brain lesion

Table 1. Baseline characteristics of study patients.

| | Total ($n = 183$) | Non-DNS Group ($n = 129$) | DNS Group ($n = 54$) | $p$ Value |
|---|---|---|---|---|
| Age (years) | 38.0 (28.0–53.0) | 37.0 (27.0–49.0) | 44.0 (29.8–57.0) | .081 |
| Male (%) | 110 (60.1%) | 75 (58.1%) | 35 (64.8%) | .414 |
| Source of CO | | | | .878 |
| Stoves (Charcoal) (%) | 131 (71.6%) | 91 (70.5%) | 40 (74.1%) | |
| Water heaters (%) | 27 (14.8%) | 20 (%15.5) | 7 (13.0%) | |
| Household gas (%) | 20 (10.9%) | 13 (10.1%) | 7 (13.0%) | |
| Others (%) | 5 (2.7%) | 5 (3.9%) | 0 (0.0%) | |
| Past history | | | | |
| Hypertension | 31 (16.9%) | 17 (13.2%) | 14 (25.9%) | .036 |
| Diabetes mellitus | 21 (11.5%) | 13 (10.1%) | 8 (14.8%) | .359 |
| Coronary artery disease | 4 (2.2%) | 2 (1.6%) | 2 (3.7%) | .583 |
| Hyperlipidemia | 5 (2.7%) | 4 (3.1%) | 1 (1.9%) | 1.000 |
| Smoking | 63 (34.4%) | 41 (31.8%) | 22 (40.7%) | .245 |
| Duration of CO exposure (hours) | 3.5 (2.0–6.0) | 2.5 (1.6–4.5) | 7.3 (4.5–9.1) | <.001 |
| Time interval between termination of CO exposure and arrival at the ED (hours) | 2.0 (1.0–2.8) | 2.0 (1.5–2.8) | 1.8 (1.0–2.4) | .142 |
| Time interval between arrival at the ED and MRI scan (minutes) | 121.0 (87.0–184.0) | 119.0 (91.5–165.0) | 154.5 (86.0–214.3) | .309 |
| Glasgow coma scale at presentation | 14.0 (8.0–15.0) | 15.0 (12.5–15.0) | 8.0 (7.0–14.0) | <.001 |
| Initial vital signs | | | | |
| Systolic blood pressure (mmHg) | 122.0 (109.0–134.0) | 124.0 (110.0–134.5) | 118.5 (108.0129.0) | .122 |
| Diastolic blood pressure (mmHg) | 76.0 (65.0–103.0) | 74.0 (65.0–80.0) | 79.0 (63.0–84.0) | .335 |
| Heart rate (beats/min) | 94.3 ± 14.4 | 93.2 ± 14.9 | 96.9 ± 12.8 | .105 |
| Initial laboratory findings | | | | |
| COHb (%) | 28.2 ± 9.9 | 27.7 ± 9.8 | 29.4 ± 10.1 | .316 |
| Netrin-1 (pg/mL) | 578.1 (413.8–722.0) | 626.0 (505.9–755.6) | 391.5 (263.0–550.5) | <.001 |
| Hemoglobin (g/dL) | 14.1 (12.3–16.0) | 14.0 (12.5–15.6) | 14.6 (11.5–16.5) | .495 |
| WBC count (x $10^3$/mm3) | 10.8 (8.3–15.6) | 9.9 (7.9–14.4) | 13.2 (10.4–19.5) | <.001 |
| Creatinine (mg/dL) | 0.87 (0.75–1.05) | 0.86 (0.75–1.04) | 0.90 (0.78–1.29) | .130 |
| Lactate (mmol/L) | 2.5 (1.4–4.4) | 2.3 (1.4–3.7) | 3.0 (1.8–5.5) | .026 |
| Arterial pH | 7.39 (7.35–7.43) | 7.39 (7.36–7.44) | 7.38 (7.34–7.41) | .099 |
| Arterial $HCO3^-$ (mmol/L) | 20.0 (18.5–23.0) | 20.7 (18.2–23.0) | 19.3 (18.7–21.3) | .238 |
| HBOT | 116 (63.4%) | 79 (61.2%) | 37 (68.5%) | .351 |
| Acute brain lesion on DW-MRI | 56 (30.6%) | 17 (13.2%) | 39 (72.2%) | <.001 |
| Duration of hospitalization (days) | 3 (1–4) | 2 (1–3) | 3 (1–4) | .576 |

Categorical variables are expressed as numbers (%) and continuous variables are expressed as means ± standard deviations or medians (interquartile ranges), as appropriate. DNS: Delayed neurological sequelae; CO: carbonmonoxide; COHb: carboxyhemoglobin; WBC: white blood cell; HBOT: hyperbaric oxygen therapy; DW-MRI: diffusion-weighted magnetic resonance imaging; Household gas: natural gas.

on DW-MRI (AOR: 8.24, 95% CI: 2.37–28.58) were independent predictors of DNS (Table 2).

A serum netrin-1 level below 432 pg/mL (cut-off value) was found to have 68.5% (95% CI: 54.4%−80.5%) sensitivity and 86.0% (95% CI: 78.8%−91.5%) specificity in the prediction of the development of DNS (Figure 2 and Table 3). In the prediction of DNS development, the sensitivity and specificity associated with the presence of an acute brain lesion on DW-MRI were 72.2% (95% CI: 58.4% −83.5%) and 86.8% (95% CI: 79.7%−92.1%), respectively (Table 3).

## Discussion

In this study, DNS developed in 29.5% of our patients, which is consistent with previous research [9,25]. We found that serum netrin-1 was an important independent predictor of DNS. We showed that the patients with serum netrin-1 levels less than 432 pg/mL had an eight-fold greater risk of developing DNS in the future compared to those with higher serum netrin-1 levels. In the prediction of DNS development, the sensitivity and specificity of serum netrin-1 were 68.5% and 86.0%, respectively.

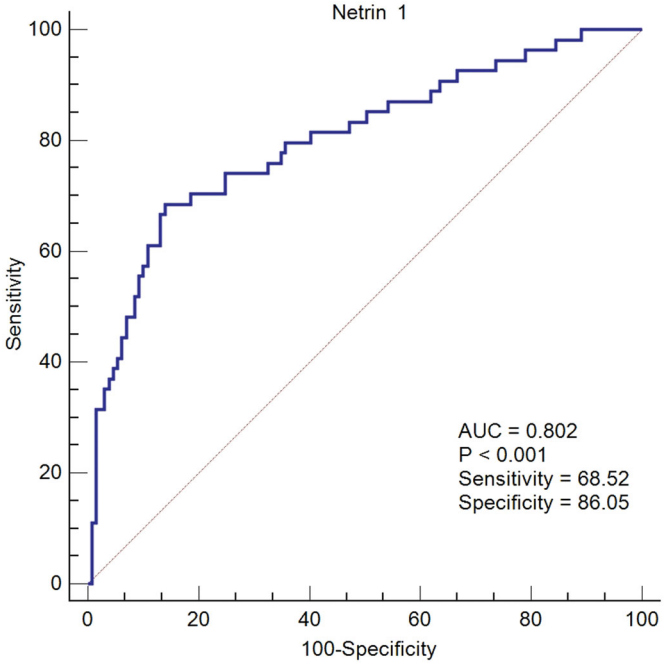


Figure 2. Receiver operating characteristic curves of serum netrin-1 level for predicting DNS. DNS: delayed neurological sequelae.

In the United States alone, the annual loss of earnings due to DNS development after acute unintentional CO poisoning was estimated to be one billion dollars [2]. Previous studies have shown that up to 45% of patients may develop DNS after acute CO poisoning [28,29]. Therefore, patients with acute CO poisoning require special attention and follow-up in terms of DNS development, even if they do not show any neurological deficit at discharge. The prediction of patients with a high risk of developing DNS is crucial in clinical practice, especially for follow-up decision making and treatment planning. Numerous studies have been conducted on blood markers (e.g., neuron-specific enolase, copeptin, troponin, COHb, and S100B) to assess the likelihood of DNS development [26,30–33]. To date, no marker has been shown to reliably detect the possibility of DNS development. However, in our study, we demonstrated that serum netrin-1 may be a good screening test with high specificity (86%) to identify patients with a high risk of developing DNS.

There are many studies on the relationship between cerebrovascular diseases and serum netrin-1 levels [16–18,34]. In these studies, it has been reported that serum netrin-1 may be a prognostic marker, especially for neurological sequelae that develop after ischemic stroke [16,17]. To the best of our knowledge, there is only one study that examined the relationship between serum netrin-1 levels and CO poisoning [35]. In that study, the diagnostic value of serum netrin-1 level in CO poisoning and its relationship with acute neurotoxicity were evaluated. The authors compared CO poisoning cases with healthy controls and observed significantly higher serum netrin-1 levels in the patient group. In the same study, the patients with and without acute neurological findings at the time of presentation were compared, and the serum netrin-1 levels did not significantly differ between these two groups. However, the authors did not specify whether the acute neurological findings were transient or permanent. In addition, they reported that they did not follow up the patients in the study in terms of the development of acute persistent neurological sequelae or DNS [35]. By contrast, we followed up all patients included in our study for six weeks

Table 2. Predictors of delayed neurological sequelae as determined by univariate and multivariate logistic regression analysis.

| | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| Variables | OR (95% CI) | *p* Value | Adjusted OR (95% CI) | *p* Value |
| Age (years) | 1.02 (0.99–1.04) | .080 | | |
| Hypertension | 2.30 (1.04–5.10) | .039 | | |
| Duration of CO exposure (hours) | 1.69 (1.44–1.97) | <.001 | 1.96 (1.49–2.56) | <.001 |
| GCS at presentation | 0.83 (0.76–0.89) | <.001 | 0.81 (0.68–0.97) | .019 |
| COHb (%) | 1.02 (0.98–1.05) | .32 | | |
| Netrin-1 (pg/mL) | 0.99 (0.98–0.99) | <.001 | | |
| Netrin-1 < 432 (pg/mL) | 12.33 (5.80–26.20) | <.001 | 8.02 (2.45–26.20) | .001 |
| WBC count (x $10^3$/mm3) | 1.15 (1.08–1.24) | <.001 | | |
| Creatinine (mg/dL) | 4.50 (1.57–12.92) | .005 | | |
| Lactate (mmol/L) | 1.17 (1.03–1.34) | .020 | | |
| Arterial $HCO_3^-$(mmol/L) | 0.98 (0.89–1.08) | .739 | | |
| Acute brain lesion on DW-MRI | 17.13 (7.82–37.52) | <.001 | 8.24 (2.37–28.58) | .001 |
| Duration of hospitalization (days) | 0.96 (0.82–1.12) | .591 | | |

OR: Odds ratio; CO: carbonmonoxide; GCS: glasgow coma scale; COHb: carboxyhemoglobin; WBC: white blood cell; DW-MRI: diffusion-weighted magnetic resonance imaging.

Table 3. Diagnostic performances of serum netrin-1 and diffusion-weighted magnetic resonance imaging for the diagnosis of DNS.

| | Clinical outcome | | |
|---|---|---|---|
| DW-MRI finding | DNS, present (+) | DNS, absent (−) | Total |
| Acute brain lesion on DW-MRI (+) | 39 | 17 | 56 |
| Acute brain lesion on DW-MRI (-) | 15 | 112 | 127 |
| Total | 54 | 129 | |
| Sensitivity (95% CI) | 72.2% (58.4–83,5) | | |
| Specificity (95% CI) | 86.8% (79.7–92.1) | | |
| Positive predictive value (95% CI) | 69.6% (58.8–78.6) | | |
| Negative predictive value (95% CI) | 88.2% (82.9–92.0) | | |
| Positive likelihood ratio (95% CI) | 5.5 (3.4–8.8) | | |
| Negative likelihood ratio (95% CI) | 0.3 (0.2–0.5) | | |
| Netrin- 1 levels | | | |
| Netrin-1 < 432 (pg/mL) | 37 | 18 | 55 |
| Netrin-1 > 432 (pg/mL) | 17 | 111 | 128 |
| Total | 54 | 129 | |
| Sensitivity (95% CI) | 68.5% (54.4–80.5) | | |
| Specificity (95% CI) | 86.0% (78.8–91.5) | | |
| Positive predictive value (95% CI) | 67.3% (56.3–76.6) | | |
| Negative predictive value (95% CI) | 86.7% (81.4–90.7) | | |
| Positive likelihood ratio (95% CI) | 4.9 (3.1–7.8) | | |
| Negative likelihood ratio (95% CI) | 0.4 (0.2–0.5) | | |

DW-MRI: Diffusion-weighted magnetic resonance imaging; DNS: delayed neurological sequelae.

and found that the serum netrin-1 levels were significantly lower in patients in the DNS group compared to those that did not develop this condition. The underlying mechanism of the relationship between low serum netrin-1 levels and acute CO poisoning is not clear. Hypoxemic brain damage, inflammatory reactions, and apoptosis are known to play a role in the pathogenesis of DNS [1,4]. Previous studies have shown that netrin-1 has anti-inflammatory and anti-apoptosis effects on neurons [12–14]. In addition, an experimental study showed that netrin-1 infusion *via* the intracerebroventricular route reduced neuronal loss and apoptosis in rats with ischemic brain damage [36]. Furthermore, high serum netrin-1 levels were reported protective against neurological sequelae after ischemic stroke [16]. Due to the mechanisms mentioned above, high serum netrin-1 levels may have also played a protective role against DNS development in our study.

The COHb level at the time of presentation has a key diagnostic value in CO poisoning. However, in terms of prognosis, this parameter does not play a major role [25]. Numerous studies have reported that the COHb level has no predictive value for the development of DNS [9,25,30,33]. In addition, low and chronic exposure to CO has been reported to cause permanent neurological and cognitive deficits [1]. Similar to previous studies, our findings showed that the COHb level was not an independent risk factor for DNS development. There are several possible explanations for the lack of a clear relationship between COHb levels and DNS. CO poisoning causes the triggering of inflammation in the brain, which may be independent of the COHb level and hypoxia degree. Hypoxia in CO poisoning can cause brain damage, but it may also be low COHb levels that trigger inflammation, leading to this damage. In addition, COHb levels may not need to be very high to trigger multiple chemical cascades that result in damage, such as lipid peroxidation, microglia activation, and activation of apoptosis [1,7].

Jeon et al. [9] reported that DW-MRI could be used as a screening method to identify patients with a high risk of developing DNS. The authors found that DW-MRI predicted DNS with 75% sensitivity and 90% specificity. In another study, Moon et al. determined that acute brain lesions detected on DW-MRI at presentation were an independent predictor of DNS [23]. The findings of our study support the results of both of these studies.

## Limitations

Our study has certain limitations. First, it had a single-center design, and therefore the generalization of our findings to other patient populations is limited. Second, since our study only included patients with unintentional CO poisoning, our results cannot be generalized to all CO poisoning cases. Third, the serum netrin-1 levels were measured only once, at the time of presentation to ED; thus, we were not able to examine the relationship between the changes in the serum netrin-1 level and DNS. However, serum netrin-1 levels in humans have been reported to be relatively stable up to 60 days [16,34]. Finally, the pre-hospital treatment of patients was not considered. For example, patients referred to the ED by ambulance had already received 100% oxygen through a non-rebreather face mask before reaching ED. However, those that present to the ED themselves do not receive any pre-hospital treatment. The difference between the two cases may have affected our results.

## Conclusions

We determined that initial serum netrin-1 was an independent predictor of DNS development. Initial serum netrin-1 and DW-MRI results can assist in the early detection of patients at risk for developing DNS. Further research is needed to validate our results.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Ethics committee approval

This study protocol was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki and Good Clinical Practices.

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