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Diagnostic Value of Parameters Related to White Blood Cell Counts for Troponin I Elevation in CO Poisoning

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Abstract
To assess myocardial injury related to acute carbon monoxide (CO) poisoning, serial troponin I is measured in patients not presenting with troponin I elevation. This retrospective study investigated whether parameters related to white blood cell (WBC) counts (total and differential WBC counts, neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio) improved predictive accuracy for troponin I elevation (> 0.04 ng/ml) in patients not presenting with evidence of myocardial injury. Serial parameters, troponin I values, and clinical courses were collected in 241 patients. Troponin I was elevated in 33 (13.7%) patients after hospitalization. The median lag times to troponin I elevation in patients with undetectable and detectable troponin I (0.015 ng/ml ≤ troponin I ≤ 0.04 ng/ml) at presentation were 5.9 h and 3.0 h, respectively. Patients with troponin I elevation after presentation had higher total WBC and neutrophil counts and NLRs and a lower lymphocyte count during the first 4 h after presentation than patients without troponin I elevation during hospitalization. Total WBC count, neutrophil count, and log NLR at presentation were selected as independent predictive factors for troponin I elevation after presentation. However, only the neutrophil count and log NLR at presentation improved the predictive accuracy in combination with clinical parameters compared with that achieved with a predictive model including only clinical parameters. The optimal cut-off neutrophil count and NLR were 5.21×10^3 /uL and 4.02, respectively. The total neutrophil count and NLR, which are widely available and inexpensive parameters obtained in the emergency department (ED), are promising screening tools for predicting the risk of troponin I elevation in patients without evidence of myocardial injury-related acute CO poisoning at presentation.

Keywords Carbon monoxide · Lymphocyte · Neutrophil · Poisoning · Troponin I

Introduction

Carbon monoxide (CO) poisoning is responsible for nearly 15000 emergency department (ED) visits and 500 deaths each year in the United States [1]. CO poisoning is unquestionably the leading cause of intentional poisoning death in some Asian countries [1].

The heart and brain, which have the highest demand for oxygen, are more vulnerable to CO poisoning [1], and up to one-third of patients with moderate-to-severe CO poisoning present with myocardial injury [2]. The clinical spectrum of myocardial injury after CO poisoning encompasses cardiomyopathy, angina attack, myocardial infarction, arrhythmias, heart failure, cardiogenic shock, and sudden death [1]. In addition, patients with myocardial injury have a higher incidence of extracardiac complications, such as burning, pneumonia, and acute kidney injury, during hospitalization [3]. Additionally, myocardial injury is associated with increased short-term outcomes (in-hospital death or neurological sequelae at discharge) [4] and long-term mortality, especially mortality due to cardiovascular causes [2]. Therefore, the assessment of myocardial injury in CO-poisoned patients at the ED is emphasized as an essential process for predicting clinical outcomes.

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Single-photon emission tomography (SPECT) has been the technique of choice for diagnosing myocardial involvement after CO poisoning, whereas troponin is now widely acknowledged as the mainstay tool for screening myocardial injury after CO poisoning. Elevated troponin I levels exceeding the 99th percentile of a normal reference population are accepted as indicative of myocardial injury with necrosis regardless of the underlying pathology [5].

However, 7.3–27.9% of patients with myocardial injury after CO poisoning did not have elevated troponin I at presentation [3]. The value of troponin I was relatively high in patients with myocardial injury diagnosed with scintigraphy in serial measurements over the first 12 h after presentation, but the value of troponin I at presentation did not differ regardless of the presence of myocardial injury diagnosed with scintigraphy [6]. Together, these clinical studies suggest that patients with CO poisoning should routinely undergo serial measurement of troponin I for the diagnosis of myocardial injury when troponin I is not elevated at presentation.

The cost for troponin I testing is higher than that of routine laboratory tests in the ED, and serial measurement of troponin I within 24 h in patients without evident acute coronary syndrome (myocardial infarction or unstable angina) is not covered by health insurance in South Korea. Furthermore, no guidelines exist concerning how much later and how many times troponin measurement should be repeated in patients to assess myocardial injury related to CO poisoning.

In addition to hypoxic damage, generation of carboxymyoglobin, mitochondrial inhibition, and increased free radical production contribute to myocardial injury after CO poisoning. Inflammation also plays an important role in the development of myocardial injury during CO poisoning. The inflammatory reaction developed at the acute stage of CO poisoning and its effects are long lasting after the initial CO poisoning [7]. White blood cells (WBCs), which can be routinely measured and whose counts are affordable at the ED, are major mediators of inflammation. Total and differential WBC counts, as biomarkers of inflammation, are associated with cardiovascular disease risk in healthy Asian and Western populations [8, 9] and in patients with heart failure [10]. Currently, the neutrophil-to-lymphocyte ratio (NLR) and monocyte-to-lymphocyte ratio (MLR), which can be obtained simply based on differential WBC counts, have emerged as new inflammatory biomarkers for assessing cardiovascular disease severity in patients with other medical diseases [11, 12].

However, no studies exploring the predictive value of parameter-related WBC counts (total and differential WBC counts, NLR, and MLR) for assessing myocardial injury in CO poisoning have been published in English.

This study aimed to investigate whether these easy and inexpensive parameters related to WBC counts improve the predictive accuracy of troponin I elevation, which is indicative of myocardial injury, after presentation in acutely CO-poisoned patients without evidence of myocardial injury at presentation. In addition, after investigating the temporal dynamics of troponin I and parameters related to WBC counts, we chose the optimal time to obtain selected parameters to predict troponin elevation after presentation.

Method

Study Design and Setting

This investigation was a retrospective, observational study performed via chart review at a single academic tertiary care center with an annual ED census of 40000 patients. The study design was approved by the Institutional Review Board of Chonnam National University Hospital.

Participant Selection

The inclusion criteria for this study were as follows: patients 18 years or older who presented to our ED with acute CO poisoning and did not show elevated troponin I (> 0.04 ng/ml) or ischemic electrocardiogram (ECG) changes at presentation between January 2014 and July 2018. CO poisoning was defined as a history of exposure to CO and an initial carboxyhemoglobin (COHb) level exceeding 5% (10% in smokers) at our ED or the primary hospital. Ischemic ECG changes included new ST-segment elevation (≥ 1 mm), depression (≥ 0.5 mm), or T waver inversion (≥ 2 mm). The exclusion criteria were as follows: pregnancy; a history of an altered mental state before the CO poisoning event; a history of coronary arterial disease or other heart disease, such as valvular disease or rhythm disease; a history of autoimmune disease, liver cirrhosis, hematological disorder, cancer, or use of immunosuppressants; death upon arrival or at the scene; mixed poisoning with other drugs; discharge against the doctor's orders; transfer before the final outcome was determined; and treatment using a protocol that did not adhere to our hospital's guidelines for CO poisoning (Fig. 1).

Our hospital's treatment guidelines for acute CO poisoning recommend the initiation of normobaric oxygen (NBO) or hyperbaric oxygen (HBO) therapy according to the presence of HBO indicators. The indicators for HBO in non-pregnant patients at our institution include the presence of any neurological deficits, including loss of consciousness, seizure, and a COHb level greater than 25%, regardless of symptoms. If the symptoms do not resolve after one session of HBO, an additional session is provided. The maximum number of HBO sessions within 24 h after presentation is

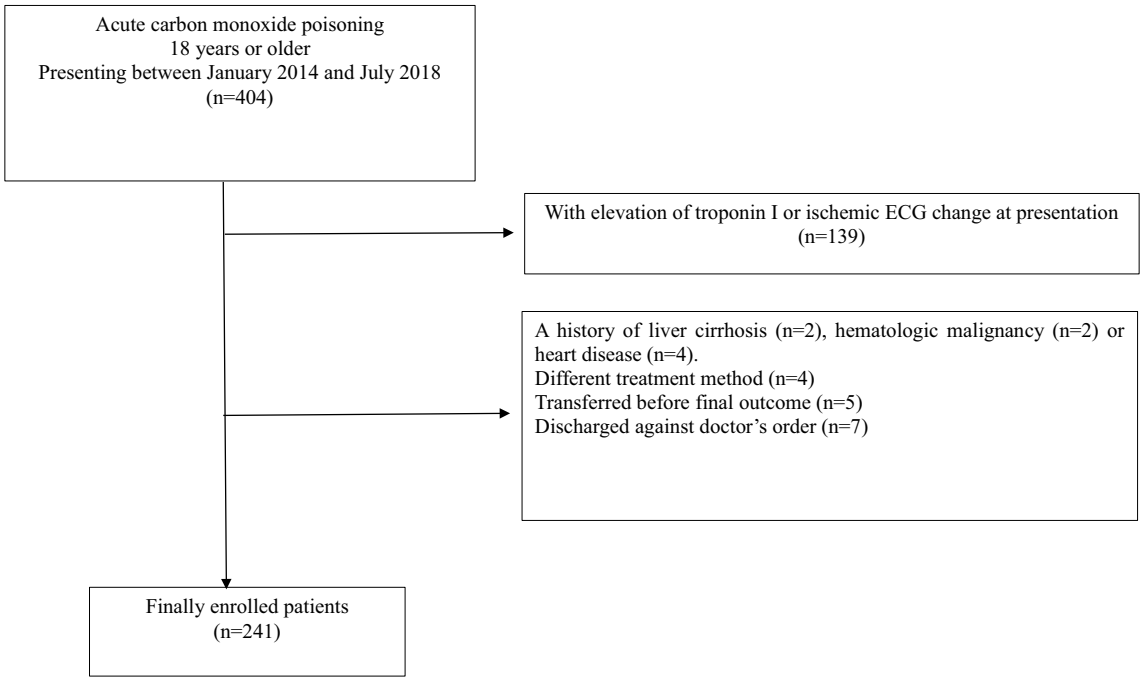

Fig. 1 Algorithm for subject selection

three. HBO therapy consists of 100% oxygen with increasing absolute pressures (from 1.0 ATA to 2.8 ATA) for 20 min, 2.8 ATA for 40 min, and decreasing absolute pressures (from 2.8 ATA to 1.0 ATA) for 20 min. When a patient with indicators for HBO requires mechanical ventilation (MV) or a vasopressor infusion due to refractory hypotension, he or she is treated with MV at a FiO_2 level of 1.0 instead of HBO.

Methods and Measurements

The following data were directly collected from electronic medical records (EMR): age, sex, laboratory results, and hospital duration. The following information was collected by reviewing patient medical records: comorbidities, source of CO, duration of exposure to CO, intentionality of exposure to CO, time interval from the last exposure to CO to arrival at our ED, Glasgow Coma Scale (GCS) score and vital signs at presentation, results of brain diffusion-weighted imaging (DWI) at presentation, corrected QT (QTc) interval at presentation, presence of indicators for HBO, time interval between arrival at the ED and initiation of the first HBO, number of HBO sessions during the first 24 h of admission, use of MV support regardless of cause, GCS at discharge, and in-hospital mortality.

Because troponin I levels peaked at 4.5–18.5 h after presentation in patients with myocardial injury diagnosed at any time during hospitalization after CO poisoning [3], and because the predictor should precede troponin I elevation, parameters related to WBC counts were collected during the first 4 h after presentation. To evaluate the temporal dynamics of parameters related to WBC counts after CO poisoning, parameters during the first 4 h were categorized as follows: at presentation and at presentation < parameters ≤ 4 h after presentation. The late category was defined as parameters obtained at 4 h after presentation. If multiple measurements were performed during this period, the highest level was used. NLR and MLR were calculated as follows: NLR = neutrophil count/lymphocyte count and MLR = monocyte count/lymphocyte count.

The corrected QT interval during ECG was automatically measured by the ECG system (MAC 5500, GE Healthcare), and the prolongation of QTc was defined as an interval ≥ 470 ms regardless of gender [13].

Troponin I Analyses

Venous blood was withdrawn and immediately sent to the emergency laboratory room. Serum troponin I levels were measured via a one-step enzyme immunoassay using a Dimension Vista® system (Siemens, Munich, Germany) with an analytical range of 0.015–40 ng/mL. The 99th percentile upper limit of the normal reference population was 0.04 ng/mL with a 10% coefficient of variation according to the manufacturer. Troponin I levels exceeding the 99th percentile of the normal reference population (troponin I > 0.04 ng/ml) were defined as elevated troponin I, which is indicative of myocardial injury. A troponin I level < 0.015 ng/mL was accepted as undetectable in this

study. To elucidate the temporal dynamics of troponin I, the lag time from presentation to troponin I elevation and the lag time to the peak troponin I were collected. These lag times were used to determine the optimal time to obtain the predictive parameters, which should precede the troponin I elevation.

Data Analysis

Patients were divided into two groups according to the troponin I elevation after presentation: the elevation group vs the non-elevation group. The baseline patient characteristics are presented as frequencies for categorical variables and as medians and interquartile ranges for continuous variables. For continuous variables, Student's t-test or the Mann–Whitney test was used for two-group comparisons according to normality, which was tested using the Shapiro–Wilk test. For categorical variables, the Chi-square test or Fisher's exact test was used. To determine whether parameters related to WBC counts significantly improved the predictive accuracy of troponin I elevation after acute CO poisoning, univariate and multivariate logistic analyses were performed. The first multivariate logistic model was applied with significant univariate predictors, while the second model was applied with univariate predictors and the total WBC count (model 2). The total WBC count was replaced by differential WBC counts in subsequent multivariate analyses (model 3 including univariate predictors, and neutrophil, lymphocyte, and monocyte counts) or log NLR and log MLR (model 4 including univariate predictors, log NLR, and log MLR). For the multivariate logistic analysis, the NLR and MLR were log-transformed because of their skewed distribution. To avoid collinearity, the differential WBC count itself and ratios of differential WBC counts (NLR and MLR) were entered into different models. Before modeling, if two or more univariate variables retained in the multivariate analysis were highly correlated in the linear regression, one variable was removed to avoid collinearity. Estimated odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for all significant variables. Receiver operating characteristic (ROC) curves were generated to obtain areas under the ROC curve (AUC) and 95% CIs and quantify the diagnostic value of each model. The AUCs and 95% CIs for troponin I elevation in each model were compared using the method described by DeLong et al. [14]. The optimal cut-off value of the parameter with the highest Youden's index (sensitivity + specificity − 1) and the value with fixed sensitivity (90% and 99%) for predicting troponin I elevation were evaluated using the ROC curve. A p value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS 21.0 (SPSS, Chicago, IL, USA) and MedCalc Statistical Software version 17.4.4 (MedCalc Software, Ostend, Belgium).

Results

Table 1 describes the baseline characteristics of the 241 patients who presented at a mean of 1 h after exposure to CO at our ED and did not show troponin I elevation or ischemic ECG changes at presentation. Troponin I was elevated to greater than 0.04 ng/ ml, which is the cut-off value to define myocardial injury, in 33 (13.7%) of 241 patients after hospitalization. Furthermore, 23 (69.7%) of the 33 patients in the elevation group had an undetectable troponin I level at presentation.

At presentation, patients in the elevation group were older and had a lower GCS score, arterial pH, and HCO_3^-; higher levels of COHb, creatine kinase (CK)-MB, and lactate; and had a higher prevalence of the need for HBO treatment than the non-elevation group. Hypertensive lesions on brain DWI and QTc prolongation during the initial ECG were more frequently observed in the elevation group than in the non-elevation group. During hospitalization, patients in the elevation group needed MV support more frequently than those in the non-elevation group and required more than one session of HBO treatment.

In the elevation group, patients with undetectable troponin I at presentation exhibited a significantly longer lag time to troponin I elevation (5.9 (3.4–7.7) hours after presentation) than patients with 0.015 ng/ml ≤ troponin I ≤ 0.04 ng/ml at presentation (3.0 (1.6–3.6) hours after presentation in the elevation group, p value = 0.004) (Fig. 2). Troponin I peaked at 7.4 (5.2–12.9) hours after presentation.

When comparing the temporal dynamics of parameters related to WBC counts, patients in the elevation group had higher total WBC and neutrophil counts, lower lymphocyte counts, and higher NLR values during first 4 h after presentation (Table 2). The monocyte count and MLR values were significantly higher at 4 h after presentation in patients in the elevation group than those in the non-elevation group.

Because the shortest lag time to troponin I elevation in the elevation group was 1 h, parameters at presentation were entered into multivariate analysis.

Multivariate analysis that included univariate clinical parameters (age, initial GCS, arterial HCO_3^-, COHb, CK-MB, undetectable troponin I (troponin I < 0.015 (ng/ml)), QTc interval) at presentation revealed that the best combination of predictors of troponin I elevation after presentation included COHb and undetectable troponin I (Table 3). In multivariate model 2, which included the clinical parameters and total WBC count at presentation, COHb, undetectable troponin I, and total WBC count were selected as independent predictors. The level of COHb, undetectable troponin I, and the neutrophil count in model

Table 1 Baseline characteristics of 241 patients according to the troponin I elevation after presentation with acute carbon monoxide (CO) poisoning

	Total (n = 241)	Non-elevation group (n = 208)	Elevation group (n = 33)	P value
Age (years)	41 (31–55)	40 (30–54)	47 (40–60)	0.028
Male	162 (67.2)	140 (67.3)	22 (66.7)	0.942
Co-morbidity				
Hypertension	24 (10)	20 (9.6)	4 (12.1)	0.655
Diabetes mellitus	13 (5.54)	10 (4.8)	3 (9.1)	0.312
Current smoking	86 (36.1)	77 (37.6)	9 (27.3)	0.417
Initial Glasgow coma scale (GCS)	15 (14–15)	15 (14–15)	14 (9–15)	0.003
Systolic blood pressure (mmHg)	110 (100–130)	115 (100–130)	110 (100–125)	0.281
Heart rate (/min)	88 (80–98)	88 (80–98)	90 (80–98)	0.396
Intentional exposure to CO	165 (68.5)	142 (68.3)	23 (69.7)	0.870
Source of CO				0.059
Charcoal	210 (87.1)	177 (85.1)	33 (100)	
Fire	19 (7.9)	19 (9.1)	0 (0)	
Others	12 (5)	12 (5.8)	0 (0)	
Exposure duration to CO (min)*	60 (30–120)	60 (30–120)	70 (30–180)	0.181
Interval from last exposure to arrival at ED (hr)	1 (0.5-3)	1 (0.5–2.8)	2 (0.8-3)	0.116
Laboratory findings				
Arterial pH	7.42 (7.39–7.45)	7.42 (7.39–7.45)	7.40 (7.37–7.44)	0.029
Arterial HCO_3^- (mmol/L)	22.4 (20.3–24)	22.6 (21-24.1)	20.7 (17.2–21.9)	0.000
Carboxyhemoglobin (%)	9.6 (4.9–17.9)	8.9 (4.4–16.2)	23.1 (8.1–37.3)	0.000
C-reactive protein (mg/dL)	0.6 (0.4–0.6)	0.6 (0.4–0.6)	0.6 (0.5–0.6)	0.245
Creatine kinase (U/L)	109 (81.5–158)	107 (81.8-157.3)	111 (80–225)	0.366
CK-MB (ng/ml)	1.1 (0.9–1.8)	1.04 (0.9–1.8)	1.3 (1.0-3.1)	0.014
NT-ProBNP (pg/ml)†	26.4 (12.7–64.9)	25.3 (11.7–59.5)	40.9 (14.6-133.9)	0.063
Undetectable Troponin I(< 0.015 (ng/ml))	223 (92.5)	200 (96.2)	23 (69.7)	0.000
Lactate (mmol/l)§	2.2 (1.3–3.4)	2.0 (1.3–3.2)	3.4 (1.9–7.8)	0.001
Abnormal brain diffusion-weighted imaging¶	8 (5.8)	4 (3.4)	4 (19)	0.005
QTc prolongation (≥ 470 ms)	103 (43.8)	81 (40.1)	22 (66.7)	0.004
The presence of HBO indication	152 (63.1)	121 (58.2)	31 (93.9)	0.000
Time interval from first HBO after arrival at ED (mins)	60 (43–90)	60 (43–90)	60 (40-97.8)	0.956
The number of HBO session during first 24 h				0.001
Two	9 (3.8)	6 (2.9)	3 (9.7)	
Three	1 (0.4)	1 (0.5)	0 (0)	
Outcome				
The need of mechanical ventilation support	6 (2.5)	2(1.0)	4 (12.1)	0.000
GCS ≤ 14 at discharge	2 (0.8)	1 (0.5)	1 (3)	0.134
Hospitalization (days)	2 (1–3)	2 (1–2)	3 (2-6.5)	0.000

Exposure duration to CO (min)*: the information concerning exposure duration to carbon monoxide was available for 169 patients

NT-ProBNP (pg/ml)†: the level of N-terminal pro-brain natriuretic peptide (NT-proBNP) was available for 233 patients

Lactate (mmol/l) §: the level of serum lactate was available for 236 patients

Abnormal brain diffusion-weighted imaging¶: brain DWI was available for 138 patients

3 (including clinical parameters, neutrophils, monocytes, and lymphocytes), and the level of COHb, undetectable troponin I, and log NLR in model 4 (including clinical parameters, log MLR, and log NLR) were selected as significant predictors.

To determine the best predictor of troponin I elevation after presentation, the AUCs of each model were compared (Fig. 3). Model 4, including log NLR at presentation, and model 3, including the neutrophil count, had a significantly higher AUC than model 1, which included only clinical

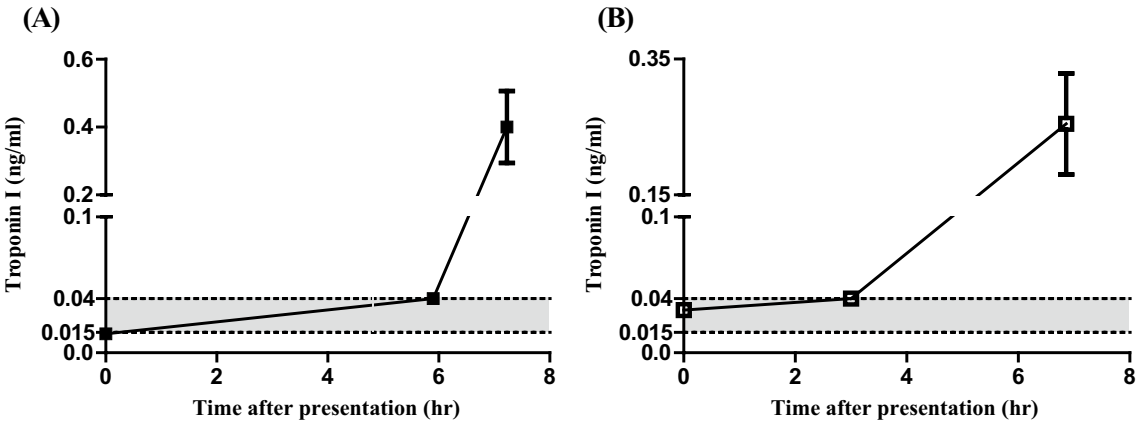

Fig. 2 Temporal dynamics of troponin I in elevation group. **a** The lag time to troponin I elevation and peak troponin I in patients with undetectable troponin I (< 0.015 ng/ml) at presentation was 5.9 (3.4–7.7) hours and 7.6 (5.9–13.5) hours after presentation, respectively. **b** The lag time to troponin I elevation and peak troponin I in patients with troponin I (0.015 ≤ troponin I ≤ 0.04 ng/ml) was 3.0 (1.6–3.6) hours and 6.7 (4.9–8.3) hours after presentation, respectively. *Gray area indicated the detectable troponin I, which is less than 99th percentile upper limit of the normal reference population, indicative of myocardial injury

Table 2 Serial measurement of parameters according to troponin I elevation after presentation

	At presentation			At 4 h after presentation[a]		
	Non-elevation group (n = 208)	Elevation group (n = 33)	P value	Non-elevation group (n = 208)	Elevation group (n = 33)	P value
Total WBCs ($\times 10^3$/mm^3)	8.6 (6.7–10.9)	10.5 (8.53–13.52)	< 0.001	8.7 (7.05–10.9)	9.2 (8.55–12.8)	0.039
Lymphocyte ($\times 10^3$/uL)	2.22 (1.59–3.02)	1.71 (1.25–2.79)	0.044	2.06 (1.6–2.52)	1.75 (1.36–2.48)	0.046
Monocyte ($\times 10^3$/uL)	0.49 (0.38–0.67)	0.49 (0.42–0.63)	0.612	0.5 (0.35–0.63)	0.58 (0.46–0.94)	0.014
Neutrophil ($\times 10^3$/uL)	5.34 (3.75–7.48)	7.84 (5.85-10.0)	< 0.001	5.63 (4.22–8.24)	6.82 (6.02–9.51)	0.005
NLR[b]	2.22 (1.39–3.88)	4.49 (2.49–7.06)	< 0.00	2.66 (1.84–4.30)	4.16 (2.89–6.99)	0.001
MLR[c]	0.22 (0.16–0.31)	0.24 (0.17–0.48)	0.090	0.24 (0.17–0.32)	0.32 (0.25–0.51)	< 0.001

[a]At 4 h after presentation: parameters were collected at time interval from presentation to 4 h after presentation

[b]NLR was the ratio of neutrophil to lymphocyte

[c]MLR was the ratio of monocyte to lymphocyte

parameters. However, models 3 and 4 had comparable predictive accuracies for troponin I elevation. The optimal cut-off values for the neutrophil count and NLR value at presentation for troponin I elevation after presentation were 5.21×10^3 /uL and 4.02, respectively (Table 4).

Discussion

The main findings of our study were that both the neutrophil count alone at presentation and the NLR at presentation improved the predictive accuracy of troponin I elevation when used in combination with clinical parameters in patients who did not show troponin I elevation or ischemic ECG changes at presentation. As the neutrophil count alone and the NLR are advantageously cost effective and easily attainable at the ED, the results of this study allow for easy predictive decision making with regard to the risk of troponin I elevation (which defines myocardial injury) at the ED and reducing the medical expense waste resulting from indiscrete serial measurements of troponin I. Because the optimal time points for obtaining the neutrophil counts and NLR were determined based on the temporal dynamics of troponin I, these study results should be practically useful in the clinical field. In addition, the fact that 69.7% of the patients in the elevation group had undetectable troponin I at presentation in this study emphasized the need for biomarkers to stratify the risk of troponin I elevation and the importance of the findings of this study.

Acute CO poisoning leads to degranulation of intravascular neutrophils, which induces generation of reactive oxygen species and catalyzes lipid peroxidation in human and animal models [15]. Along with the higher neutrophil count observed over the first 4 h after presentation in the

Table 3 Multivariate logistic regression to identify independent factors for troponin I elevation after presentation

Variable	Multivariate logistic regression (Odds ratio (95% confidence interval))			
	Model 1	Model 2	Model 3	Model 4
Age	1.005 (0.977–1.035)	1.010 (0.981–1.040)	1.003 (0.974–1.033)	0.998 (0.969–1.029)
Initial Glasgow Coma Scale	0.929 (0.823–1.048)	0.952 (0.838–1.082)	0.900 (0.786–1.031)	0.899 (0.791–1.021)
Arterial HCO_3^-	0.944 (0.827–1.077)	0.924 (0.807–1.059)	0.889 (0.770–1.027)	0.884 (0.764–1.023)
Carboxyhemoglobin	1.054 (1.017–1.093)	1.041 (1.001–1.082)	1.056 (1.016–1.097)	1.055 (1.016–1.095)
Creatine kinase-MB	1.050 (0.934–1.181)	1.017 (0.903–1.145)	0.983 (0.870–1.110)	0.987(0.876–1.112)
Undetectable troponin I	0.173 (0.049–0.613)	0.175 (0.048–0.636)	0.163 (0.041–0.653)	0.157 (0.040–0.615)
QTc prolongation	0.430 (0.173–1.070)	0.482 (0.190–1.226)	0.512 (0.195–1.341)	0.525 (0.198–1.391)
Total white blood cell count		1.123 (0.995–1.269)		
Neutrophil count			1.155 (1.017–1.311)	
Monocyte count			0.853 (0.211–3.443)	
Lymphocyte count			0.663 (0.432–1.018)	
Log NLR*				2.916 (1.364–6.231)
Log MLR§				1.118 (0.437–2.859)

*NLR** neutrophil-to-lymphocyte ratio

MLR§ monocyte-to-lymphocyte ratio

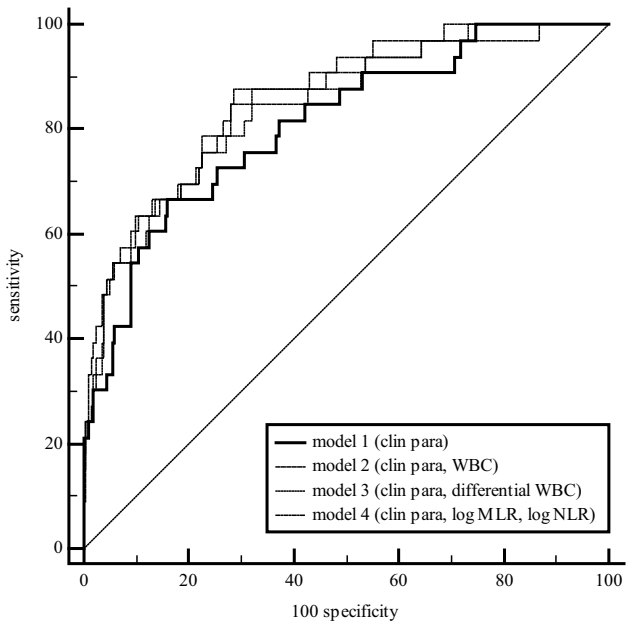

Fig. 3 Comparison of predictive accuracy of four models for troponin I elevation. The area under curves of each model were 0.808 (0.751–0.856) for model 1, 0.851 (0.799–0.894) for model 2, 0.844 (0.791–0.888) for model 3, and 0.854 (0.802–0.897) for model 4, respectively. Model 4 and model 3 had significantly higher predictive accuracy than model 1 (model 4 vs model 1 p = 0.027; model 3 vs. model 1 p = 0.048). There was no difference in predictive accuracy between model 1 and model 2

elevation group herein, a higher neutrophil count at presentation in patients with severe CO poisoning compared with that in control group patients [16], and an abrupt increase in neutrophil counts on the day of CO poisoning followed by a decrease over 14 days after presentation [17] together support the involvement of neutrophils in the pathology of CO poisoning. Because neutrophils are well-known potential biomarkers of inflammation and because inflammation is responsible for the pathomechanism of myocardial injury after CO poisoning, the significance of the neutrophil count in this study is not surprising.

The NLR is a comprehensive inflammatory index reflecting both the proinflammatory status and immunosuppression by combining neutrophils and lymphocytes. This ratio has been proven to be a useful prognostic factor in many diseases, such as neoplastic disease, stroke, and cardiovascular disease [18]. Consistent with our results, a recent Chinese study stated that the NLR upon admission was an independent factor for myocardial injury diagnosis due to troponin I upon elevation upon admission for CO poisoning [19]. However, interestingly, the performance accuracy of NLR for detecting troponin I elevation was similar to that of the neutrophil count alone. The comparable predictive performances of these two parameters at presentation might indicate that neutrophils are the main innate inflammatory cells responsible for the quick response that causes myocardial injury after CO poisoning. In contrast to neutrophils, there is a comparative paucity of data regarding the role of lymphocytes in the pathogenesis after CO poisoning. In this study, the lymphocyte count was significantly lower over first 4 h in the elevation group than in the non-elevation group. Physiological stress and the subsequent neurohormonal system activation lead to cortisol and catecholamine release, which in turn mediates lymphocytopenia in peripheral blood via lymphocyte apoptosis [20]. An increase in catecholamine was observed

Table 4 Predictive accuracy and cut-off value of neutrophil count and NLR (neutrophil-to-lymphocyte ratio) for troponin I elevation

	Area under ROC curve	Cut-off value	Sensitivity	Specificity
NLR				
Optimal cut-off value	0.724 (0.663–0.780)	4.02	66.7 (48.2–82.0)	76.9 (70.6–82.5)
At fixed sensitivity				
90%		1.593	90	29.8 (1.3–58.7)
99%		0.43	99	0.5 (0.02-1.0)
Neutrophil count				
Optimal cut-off value	0.722 (0.661–0.778)	5.21 ($\times 10^3$/uL)	87.8 (71.8–96.6)	48.1 (41.1–55.1)
At fixed sensitivity				
90%		4.182 ($\times 10^3$/uL)	90	32.7 (11.7–53.4)
99%		3.197 ($\times 10^3$/uL)	99	16.8 (8.7–30.3)

in the pericardial fluid of patients with acute CO poisoning [21]. The poor prognostic significance of lymphocytopenia during admission in acute myocardial infarction [22] and myocardial ischemic/reperfusion injury [23] was demonstrated. The role of lymphocytes in healing and repairing the damaged myocardium in the later stages was recently shown [24]. The delayed action of lymphocytes in myocardial injury might decrease the significance of lymphocytes for predicting troponin I elevation, which only determines the onset of injury during acute stage regardless of subsequent repair. Further studies on the role of lymphocytes in CO poisoning are needed.

Comparable predictive performances of two parameters, a simple neutrophil count by itself and NLR, can lead to the question of which one is better in the clinical field. NLR is more stable than the neutrophil count alone because the neutrophil count is easily affected by other diseases, such as infection and stress, or medication, which makes the change in the neutrophil count less informative [25]. However, the sensitivity of the optimal cut-off value of the NLR was lower than that of the neutrophil count alone. Although the ideal (but unrealistic) situation is to produce a test that is 100% accurate, a good alternative is to subject patients who are initially positive to a test with high sensitivity/low specificity to a second test with low sensitivity/high specificity. Simultaneous tests of the neutrophil count and NLR may exhibit increased sensitivity.

The optimal cut-off value for the NLR in this study differed from that of the NLR in a previous study [19]. This difference can be explained by the different outcomes (troponin I elevation after admission vs troponin I elevation at admission). Earlier troponin I elevation might be accompanied by more significant neutrophilia and lymphocytopenia, which was shown to lead to a higher NLR cut-off value in a previous study. This explanation was supported by the comparison of parameters related to WBC counts at presentation between patients with evident myocardial injury at presentation and patients with troponin I elevation after presentation in our hospital during study period (Supplement Table 1).

The total WBC count, which was selected in the multivariate analysis, failed to improve the predictive accuracy for troponin I elevation after presentation compared to model 1. This might be explained by the offset of change in the differential WBC counts (increased neutrophil and decreased lymphocyte) by the summation.

We demonstrated the lag times to troponin I elevation and the lag time to peak level in the elevation group. The lag time to peak (7.4 (5.2–12.9) hours) in our study tended to be shorter than that in a previous study (11.0 (4.5–18.5) hours) [3]. Cha et al. included patients with myocardial injury based on troponin I elevation or ischemic ECG changes at presentation [3], which might have indicated that severe myocardial injuries, in which troponin I was released faster, for a longer period and at higher levels, were included in their study. The lag time to troponin I elevation in this study was suggested according to the initial level of troponin I (5.9 and 3.0 h of median lag time in patients with undetectable troponin and $0.015 \leq$ troponin I ≤ 0.04 ng/ml at presentation, respectively). The lag time to troponin I elevation is more informative than that of peak troponin I for selecting the optimal time of biomarker for troponin I elevation. In addition, this lag time provides a basis for establishing guidelines for the follow-up period to assess myocardial injury using serial troponin I measurement in patients without troponin I elevation at presentation after CO poisoning. Additionally, the lag time suggested a time window for obtaining new biomarkers to replace troponin I measurement for assessing myocardial injury.

A number of previous studies demonstrated that elevated COHb levels are indicative of exposure but do not correlate with the severity of acute symptoms or neurological outcomes [26–28]. When confined to myocardial injury, COHb was associated with troponin I elevation [29]. The COHb

level has a relationship with myocardial injury, as confirmed by scintigraphy [6], and higher levels of COHb are associated with long-term development of myocardial infarction after CO poisoning [30]. In contrast, COHb was not associated with myocardial injury diagnosed using elevation of troponin I, CK, and CK-MB [2] or with myocardial injury diagnosed using troponin I and ischemic ECG findings [3]. This inconsistent association might be explained by the different definitions of myocardial injury and the different characteristics of populations analyzed (duration of exposure, space to exposure to CO, etc.). The final amount of COHb generated depends on the duration of exposure, concentration in the inspired air, and alveolar ventilation [31]. In this study, COHb was an independent predictor of troponin I elevation, and the hypoxic effect of the COHb molecule on myocytes might be the primary factor responsible for this significance.

In this study, the higher frequency of MV support in the elevation group supported previous studies showing the poor outcome of patients with myocardial injury after acute CO poisoning regardless of the time of myocardial injury diagnosis [1].

Our study has several inherent limitations. First, because of its retrospective design and low number of patients in the elevation group (n = 33), statistical bias might have been introduced, and the results might thus not be generalizable for the whole population. Additionally, confounding factors interfering with WBC counts might not have been completely excluded. Second, the 99th percentile of troponin I is influenced by age and sex [32]. However, we applied the 99th percentile supplied by the manufacturer to our patients without regard to their variability, which might have led to overestimation of the incidence of elevated troponin I. Third, the prevalence of troponin I elevation, as a marker of myocardial injury, might be underestimated due to the use of conventional immunoassays, which only allow identification of large areas of myocardial necrosis. Highly sensitive troponin I assays, which allow detection of much lower amounts of cardiac troponin in the blood, are widely implemented in the ED. Based on repeated measurements obtained over 6 h, the diagnostic performance for acute myocardial infarction was similar for both tests [33]. In this study, troponin I was measured at least twice in patients.

Conclusion

Both neutrophil counts and the NLR at presentation improved the predictive accuracy in combination with clinical parameters for detecting troponin I elevation in patients without elevated troponin I or ischemic ECG changes at presentation after acute CO poisoning. Because these parameters can easily be measured and are widely attainable in the ED, they serve as a promising screening tool for patients with a risk of myocardial injury after CO poisoning even without evidence of myocardial injury at presentation.

Compliance with Ethical Standards

Conflict of interest The authors declare that we have no conflict of interest.

References

1. Lippi, G., Rastelli, G., Meschi, T., Borghi, L., & Cervellin, G. (2012). Pathophysiology, clinics, diagnosis and treatment of heart involvement in carbon monoxide poisoning. *Clinical Biochemistry, 45*, 1278–1285.
2. Satran, D., Henry, C. R., Adkinson, C., Nicholson, C. I., Bracha, Y., & Henry, T. D. (2005). Cardiovascular manifestations of moderate to severe carbon monoxide poisoning. *Journal of the American College of Cardiology, 45*, 1513–1516.
3. Cha, Y. S., Cha, K. C., Kim, O. H., Lee, K. H., Hwang, S. O., & Kim, H. (2014). Features and predictors of myocardial injury in carbon monoxide poisoned patients. *Emerg Med J, 31*, 210–215.
4. Kao, H. K., Lien, T. C., Kou, Y. R., & Wang, J. H. (2009). Assessment of myocardial injury in the emergency department independently predicts the short-term poor outcome in patients with severe carbon monoxide poisoning receiving mechanical ventilation and hyperbaric oxygen therapy. *Pulmonary Pharmacology and Therapeutics, 22*, 473–477.
5. Thygesen, K., Alpert, J. S., Jaffe, A. S., Chaitman, B. R., Bax, J. J., Morrow, D. A., & White, H. D., ESC Scientific Document Group. Fourth universal definition of myocardial infarction (2018). *European Heart Journal*. 2018; https://doi.org/10.1093/eurheartj/ehy462.
6. Aslan, S., Uzkeser, M., Seven, B., Gundogdu, F., Acemoglu, H., Aksakal, E., & Varoglu, E. (2006). The evaluation of myocardial damage in 83 young adults with carbon monoxide poisoning in the East Anatolia region in Turkey. *Human and Experimental Toxicology, 25*, 439–446.
7. Rose, J. J., Wang, L., Xu, Q., McTiernan, C. F., Shiva, S., Tejero, J., & Gladwin, M. T. (2017). Carbon monoxide poisoning: pathogenesis, management, and future directions of therapy. *American Journal of Respiratory and Critical Care Medicine, 195*, 596–606.
8. Mochizuki, K., Miyauchi, R., Misaki, Y., Kasezawa, N., Tohyama, K., & Goda, T. (2012). Associations between leukocyte counts and cardiovascular disease risk factors in apparently healthy Japanese men. *Journal of Nutritional Science and Vitaminology, 58*, 181–186.
9. Nordestgaard, B. G., Adourian, A. S., Freiberg, J. J., Guo, Y., Muntendam, P., & Falk, E. (2010). Risk factors for near-term myocardial infarction in apparently healthy men and women. *Clinical Chemistry, 56*, 559–567.
10. Sabatine, M. S., Morrow, D. A., Cannon, C. P., Murphy, S. A., Demopoulos, L. A., DiBattiste, P. M., McCabe, C. H., Braunwald, E., & Gibson, C. M. (2002). Relationship between baseline white blood cell count and degree of coronary artery disease and mortality in patients with acute coronary syndromes: a TACTICS-TIMI 18 (treat angina with aggrastat and determine cost of therapy with an invasive or conservative strategy- thrombolysis in myocardial

infarction 18 trial) substudy. *Journal of the American College of Cardiology, 40*(10), 1761–1768.
11. Azab, B., Chainani, V., Shah, N., & McGinn, J. T. (2013). Neutrophil-lymphocyteratio as a predictor of major adverse cardiac events among diabeticpopulation: a 4-year follow-up study. *Angiology, 64*, 456–465.
12. Xiang, F., Chen, R., Cao, X., Shen, B., Liu, Z., Tan, X., Ding, X., & Zou, J. (2018). Monocyte/lymphocyte ratio as a better predictor of cardiovascular and all-cause mortality in hemodialysis patients: a prospective cohort study. *Hemodialysis International, 22*, 82–92.
13. Manini, A. F., Nelson, L. S., Skolnick, A. H., Slater, W., & Hoffman, R. S. (2010). Electrocardiographic predictors of adverse cardiovascular events in suspected poisoning. *Journal of Medical Toxicology, 6*, 106–115.
14. DeLong, E. R., DeLong, D. M., & Clarke-Pearson, D. L. (1988). Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics, 44*, 837–845.
15. Thom, S. R., Bhopale, V. M., Han, S. T., Clark, J. M., & Hardy, K. R. (2006). Intravascular neutrophil activation due to carbon monoxide poisoning. *American Journal of Respiratory and Critical Care Medicine, 174*, 1239–1248.
16. Schnittger, V., Rosendahl, K., Lind, F., & Palmblad, J. (2004). Effects of carbon monoxide poisoning on neutrophil responses in patients treated with hyperbaric oxygen. *Journal of Investigative Medicine, 52*, 523–530.
17. Lee, S. S., Choi, I. S., & Song, K. S. (1994). Hematologic changes in acute carbon monoxide intoxication. *Yonsei Medical Journal, 35*, 245–251.
18. Dong, C. H., Wang, Z. M., & Chen, S. Y. (2018). Neutrophil to lymphocyte ratio predict mortality and major adverse cardiac events in acute coronary syndrome: a systematic review and meta-analysis. *Clinical Biochemistry, 52*, 131–136.
19. Han, Y. Y., Wang, Y., Zhao, G. Q., Yang, J. L., Wang, L., & Wang, W. Z. (2018). Relationship between neutrophil-to-lymphocyte ratio and myocardial injury induced by acute carbon monoxide poisoning. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi, 36*, 362–364.
20. Thomson, S. P., McMahon, L. J., & Nugent, C. A. (1980). Endogenous cortisol: a regulator of the number of lymphocytes in peripheral blood. *Clinical Immunology and Immunopathology, 17*, 506–514.
21. Ishikawa, T., Quan, L., Michiue, T., Kawamoto, O., Wang, Q., Chen, J. H., Zhu, B. L., & Maeda, H. (2013). Postmortem catecholamine levels in pericardial and cerebrospinal fluids with regard to the cause of death in medicolegal autopsy. *Forensic Science International, 228*, 52–60.
22. Blum, A., Sclarovsky, S., Rehavia, E., & Shohat, B. (1994). Levels of T-lymphocyte subpopulations, interleukin-1 beta, and soluble interleukin-2 receptor in acute myocardial infarction. *American Heart Journal, 127*, 1226–1230.
23. Boag, S. E., Das, R., Shmeleva, E. V., Bagnall, A., Egred, M., Howard, N., Bennaceur, K., Zaman, A., Keavney, B., & Spyridopoulos, I. (2015). T lymphocytes and fractalkine contribute to myocardial ischemia/reperfusion injury in patients. *The Journal of Clinical Investigation, 125*, 3063–3076.
24. Boag, S. E., Andreano, E., & Spyridopoulos, I. (2017). Lymphocyte Communication in Myocardial Ischemia/Reperfusion Injury. *Antioxidants and Redox Signaling, 26*(12), 660–675.
25. Lin, J., Xue, B., Li, J., Xu, H., Huang, X., Yao, Z., Li, X., & Xia, J. (2017). Neutrophil to lymphocyte ratio may be a helpful marker to evaluate disease activity in NMOSD. *Neurological Sciences, 38*, 1859–1863.
26. Annane, D., Chevret, S., Jars-Guincestre, C., Chillet, P., Elkharrat, D., Gajdos, P., & Raphael, C. (2001). Prognostic factors in unintentional mild carbon monoxide poisoning. *Intensive Care Medicine, 27*, 1776–1781.
27. Cervellin, G., Comelli, I., Rastelli, G., Picanza, A., & Lippi, G. (2014). 4.Initial blood lactate correlates with carboxyhemoglobin and clinical severity in carbon monoxide poisoned patients. *Clinical Biochemistry, 47*, 298–301.
28. Hampson, N. B. (2018). Carboxyhemoglobin: a primer for clinicians. *Undersea and Hyperbaric Medicine, 45*(2), 165–171.
29. Yelken, B., Tanriverdi, B., Cetinbaş, F., Memiş, D., & Süt, N. (2009). The assessment of QT intervals in acute carbon monoxide poisoning. *Anadolu Kardiyoloji Dergisi, 9*, 397–400.
30. Kaya, H., Coşkun, A., Beton, O., Zorlu, A., Kurt, R., Yucel, H., Gunes, H., & Yılmaz, M. B. (2016). COHgb levels predict the long-term development of acute myocardial infarction in CO poisoning. *The American Journal of Emergency Medicine, 34*(5), 840–844.
31. Tikuisis, P., Kane, D. M., McLellan, T. M., Buick, F., & Fairburn, S. M. (1985). Rate of formation of carboxyhemoglobin in exercising humans exposed to carbon monoxide. *Journal of Applied Physiology, 72*, 1311–1319.
32. Bossard, M., Thériault, S., Aeschbacher, S., Schoen, T., Kunz, S., von Rotz, M., Estis, J., Todd, J., Risch, M., Mueller, C., Risch, L., Paré, G., & Conen, D. (2017). Factors independently associated with cardiac troponin I levels in young and healthy adults from the general population. *Clinical Research in Cardiology, 106*, 96–104.
33. Alazzoni, A. Al-Saleh,A., Shalash, S. A., Ye, C., Mbuagbaw, L., Thabane, L., Sanjit, S., & Jolly (2014). Performance of the high-sensitivity troponin assay in diagnosing acute myocardial infarction: systematic review and meta-analysis. *CMAJ Open, 2*(3), E199–E207.