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Hematological parameters as early predictors of delayed neurological sequelae in acute carbon monoxide poisoning

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Abstract

Introduction: Delayed neurological sequelae (DNS) are the most frequent morbidity after acute carbon monoxide (CO) poisoning. Therefore, their prevention and early prediction are the main goal of treatment. **Aim of the work:** This study aimed to investigate parameters based on peripheral complete blood cell count (CBC) as predictors of DNS in acute CO poisoning. **Methodology**: It included 67 acutely CO poisoned patients and 38 healthy volunteers. On admission, history, clinical examination and routine laboratory investigations were done. Ratios based on CBC counts were measured. Patients were followed up along 6 months for DNS signs. **Results**: The results revealed that total white blood cells (WBC) count, absolute neutrophil count & percentage and median values of neutrophil-lymphocyte ratio (NLR) & systemic immune inflammation index (SII) were significantly higher while lymphocytes percentage, absolute monocytic count and percentage were significantly lower in CO poisoned patients compared to control group. DNS-complicated patients had significantly lower total WBC count, absolute neutrophil count & percentage and significantly higher median values of NLR and SII than non-complicated. Based on receiver operating characteristic analysis (ROC), NLR and SII were significant predictors of DNS. **Conclusion**: It was concluded that, NLR and SII ratios may be helpful predictors of DNS after acute CO poisoning.

Key words Carbon monoxide poisoning, neutrophils, systemic immune inflammation index, delayed neurological sequelae, prognosis

Introduction

Carbon monoxide (CO) is a known highly toxic gas produced by incomplete combustion of organic materials, with specific properties as it is colorless, odorless, tasteless, non-irritant gas *(Wang et al., 2019)*. Acute CO poisoning remains an important cause of morbidity and mortality and represents a major public health problem *(Liao et al., 2019b)*. About 50,000 cases with 1200 deaths for non-fire CO exposures were reported annually in the United States *(Kim et al., 2019)*.

The affinity of CO for hemoglobin is 250 times much higher than that of oxygen *(Eichhorn et al., 2018)*. Therefore, CO binds to hemoglobin rapidly after exposure to form carboxy-hemoglobin (COHb) that has lower oxygen-carrying capacity leading to tissue hypoxia, oxidative stress and inflammation *(Huysal et al., 2016)*. Additionally, CO causes free oxygen radicals formation and lipid peroxidation in different body tissues causing changes in the morphology and function of those organs *(Cevik et al., 2010)*.

Brain and nervous system are the most injured organs by tissue hypoxia induced by acute CO poisoning causing delayed neurological sequelae (DNS) *(Pang et al., 2013)*. It includes many symptoms and signs (recurrent headache, delirium, amnesia, cognitive dysfunction, personality changes, psychosis, urine and fecal incontinence) (*Sönmez et al., 2018)*. After an initial clinical recovery from acute CO poisoning, DNS had been detected within 2 to 6 weeks in 3% to 40% of cases. It may resolve gradually within the first months but unfortunately it can be permanent in 25% of cases. So, early prediction and prevention of DNS after acute CO poisoning are considered nowadays the main line of treatment *(Guan et al., 2015; Jeon et al., 2018; Lin et al., 2018)*.

Although exact pathophysiology of DNS remains unclear, systemic inflammation process was found to have important role in the occurrence of DNS and fatal complications due to acute CO poisoning *(Moon et al., 2019a)*. Following CO exposure, significant increase in leukocyte sequestration in brain microvasculature was detected. Additionally, the flux of nitric oxide released from platelets increases due to CO poisoning that may stimulate heterotypic (platelet–neutrophil) aggregation *(Thom et al., 2006b; Karabacak et al., 2015*). Furthermore, leukocytosis, neutrophilia, monocytosis, and lymphocytopenia have been detected in the acute phase of clinical conditions in which oxidative stress is increased *(Kim et al., 2015)*.

Moreover, no correlation has been found between COHb level in the blood and clinical symptoms or long-term prognosis of acute CO poisoning. This suggests that other processes rather than hypoxia such as inflammation may be indicative of occurrence of DNS and later complications. In particular, laboratory markers of inflammation would be helpful for clinicians to predict DNS after acute CO poisoning *(Vezzani et al., 2016)*.

Recently, neutrophil-lymphocyte ratio (NLR), monocyte-lymphocyte ratio (MLR) and platelet-lymphocyte ratio (PLR) are new simple inflammatory biomarkers which can be obtained easily based on peripheral complete blood cell (CBC) counts. They are useful prognostic marker in various clinical conditions with inflammatory responses *(Kim et al., 2015; Moon et al., 2019b)*. Systemic immune inflammation index (SII) is a new inflammatory marker which was found to be a more objective prognostic marker as it reflects the balance between inflammatory and immune response of the body *(Hu et al., 2014; Geng et al., 2016)*.

Hyperbaric oxygen (HBO) therapy is the primary method of treatment for acute severe CO poisoning due to its beneficial effect in preventing inflammatory changes in the brain and lipid peroxidation, consequently decreasing DNS rates *(Vezzani et al., 2016; Hafez and El-Sarnagawy, 2019)*.

Although, association between systemic inflammation and DNS in acute CO poisoning was proved *(Moon et al., 2019a)* and there are multiple ratios based on peripheral CBC count available as new inflammatory prognostic markers, there are limited studies exploring the predictive value of these ratios for DNS in acute CO poisoning.

So, this study aimed to investigate whether hematological parameters based on peripheral CBC counts are associated with occurrence of DNS after acute CO poisoning and which parameters could predict them.

Patients and Methods

Study design

This prospective cohort study was carried out on all acute CO intoxicated patients who were admitted to Tanta University Poison Control Center (TUPCC) in the period from the start of November 2017 to the end of October 2018. Age and sex matched non-exposed 38 healthy volunteers (control group) were also included.

This study was performed after approval by the Research Ethics Committee (REC) of Faculty of Medicine, Tanta University (approval number: 31900/11/17). A written informed consent was explained and signed from each patient or from guardian of unconscious patients prior to the start of the study. Participants were anonymized with strict consideration of the confidentiality of personal and clinical data.

Eligibility criteria

Inclusion criteria: All patients of both sexes above 18 years with acute CO poisoning were included in this study. Diagnosis of acute CO intoxication was established based on history of CO exposure, clinical findings of poisoning (such as headache, nausea, vomiting, dizziness, malaise, alteration in consciousness level, syncope, seizures, shortness of breath, chest pain and palpitation). Elevated COHb level > 3-4% in nonsmokers or >10% in smokers confirmed the diagnosis *(Hampson et al., 2012; Rose et al., 2017)*.

Exclusion criteria: Patients with history of co-ingestion of other poisons, patients with risk factors such as pregnancy, chronic chest diseases e.g. asthma and chronic hematological diseases e.g. anemia as well as patients transferred from other hospitals were excluded. Moreover, patients with previous history of a neuropsychiatric disease or impaired cerebral perfusion and patients who had any disease that may affect white blood cells (WBC) count such as obesity, diabetes mellitus, malignancy, acute or chronic renal and liver disease, acute or chronic inflammatory disease, autoimmune disease, atherosclerotic heart diseases, valvular heart disease, heart failure, peripheral arterial disease were also excluded.

Data collection and definition of variables

Demographics (age and sex), medical and toxicological history (delay period between exposure and hospital arrival and duration of exposure) were recorded for each patient in data collection sheet. Clinical examination including Glasgow coma score (GCS), vital signs, neurological, respiratory and cardiovascular examination was done at admission for each patient.

All patients were treated by administering 100% normobaric oxygen by a non-rebreather mask upon arrival at hospital. The decision to refer patients to HBO therapy unit at Tanta University Educational Hospital was made by the treating physicians at TUPCC following the guidelines of HBO therapy for CO poisoning that were loss of consciousness on admission, neurological deficits, ischemic cardiac changes, significant metabolic acidosis and elevated COHb level ≥ 25% *(Liao et al., 2019a)*. Number of needed HBO sessions and duration of hospital stay were registered.

Blood sample and biochemical investigations

Blood samples were collected immediately after admission under complete aseptic conditions and before giving any medication for arterial blood gases and peripheral CBC counts analysis. Peripheral CBC ratios were calculated as follows: SII = platelet count × neutrophil count/lymphocyte count (Hu et al., 2014); NLR = neutrophil count/lymphocyte count; MLR = monocyte count/lymphocyte count; and PLR = platelet count/lymphocyte count.

The Rad-57 Pulse CO-Oximeter (Masimo SET Rainbow, Irvine, California, USA) depending on a multi-wave length sensor was used as a continuous and non-invasive method of measuring COHb level in blood at admission. Twelve-lead electrocardiography (ECG) was recorded for each patient at admission and corrected QT (QTc) interval was detected according to the Bazett's

formula; $QTc = QT/\sqrt{RR}$ (Normally, QTc is ≤ 440 msec) *(Postema and Wilde, 2014).*

ECG changes induced by acute CO poisoning were graded according to poisoning severity score *(Akdur et al., 2010)* into: *Minor ECG changes:* Isolated extrasystoles, sinus tachycardia (HR ≥ 100-140 in adults). *Moderate ECG changes:* Sinus tachycardia (HR =140-180 in adults), frequent extrasystoles, atrial fibrillation/flutter, AV-block I-II, prolonged QRS and QTc-time, repolarization abnormalities or myocardial ischaemia. *Severe ECG changes:* severe sinus tachycardia (HR≥180 in adults), life-threatening ventricular dysrythmias, AV block III, asystole, myocardial infarction.

Follow up for DNS

Assessment of acute CO poisoned patient's neurological and psychological outcome was done initially after patient regain stable level of consciousness. Then, all patients were invited for follow up regular visits after being discharged for detection of any manifestations that referred as DNS and patients with any detected DNS were directed to have neuropsychiatric consultation. The recommended follow up duration was at least 6 months after acute CO poisoning. Symptoms of DNS were recorded in a simple questionnaire including leading questions.

Occurrence of DNS in the included patients was confirmed by recurrence of original neurological or psychological symptoms or by development of new ones. The symptoms included one or more of the following: difficulty concentrating, lethargy, emotional liability, amnestic syndromes, dementia, cognitive impairment, personality changes, learning difficulties, psychosis, depression, Parkinsonism, apraxia, gait disturbance, urinary incontinence *(Pepe et al., 2011; Cha et al., 2018).*

Statistical Analysis

Acute CO poisoned patients were divided into two groups according to appearance of DNS after co poisoning: DNS-complicated and non-complicated patients. Collected data were organized and entered to the computer using SPSS (Statistical Package for Social Science) program for statistical analysis (version 22). Data were entered as numerical or categorical, as appropriate. Kolmogorov-Smirnov test of normality revealed significance in the distribution of some variables, so the non-parametric statistics were adopted. Minimum, maximum, median and inter-quartile range (IQR) were calculated. Chi-Squared test (Monte-Carlo corrected) for NxM table. Receiver operating characteristic (ROC) analysis was generated from the data for a discriminative cut-off value for predicting the occurrence of DNS. Sensitivity and specificity were also calculated using Medcalc software. Area under ROC curve (AUC) was graded as follows: 0.90-1 = excellent; 0.80-0.90 = good; 0.70-0.80 = fair; and 0.60-0.70 = poor.

Results

Demographics, vital signs and QTc interval of acute CO poisoned patients were compared to control group as shown in table (1). Age and sex were comparable in both groups with no statistical difference. Blood pressure (both systolic and diastolic) and oxygen saturation were significantly lower while QTc interval was significantly longer in CO poisoned patients.

Concerning hematological parameters (table 2), data obtained by mann-whitney test revealed that haemoglobin level, total WBC count, absolute neutrophil count and percentage were significantly higher while lymphocytes percentage, absolute monocytic count and percentage were significantly lower in CO poisoned patients than control group. Moreover, median values of NLR and SII were significantly higher in CO poisoned patients (3.35 and 869.6 respectively) when compared with control group (1.92 and 488.8 respectively) while; MLR and PLR did not show any statistical difference between them.

DNS had developed in 33 patients (53.2%). The frequency of detected symptoms within DNS-complicated patients were recurrent headache and memory loss (27.3% each), difficult speech and concentration deficit (24.2% each), personality changes (18.2%), insomnia and gait disturbance (9.1% each).

Comparisons of demographics, admission data, vital signs, QTc, ECG severity grading, COHb level, hospital stay and number of needed HBO sessions between DNS-complicated and non-complicated patients were done (Table 3,4). DNS-complicated patients had significantly lower initial GCS and oxygen saturation and higher COHb level on admission, length of hospital stay and number of needed HBO sessions than non-complicated ones.

Table (5) showed that DNS-complicated patients had significant higher median values of total WBC count, absolute neutrophil count and percentage and lower median value of lymphocytes percentage than non-complicated ones. NLR and SII median values were significantly higher in DNS-complicated patients (4.85 and 1112.4 respectively) than non-complicated ones (2.51 and 730.3 respectively) while; MLR and PLR did not show any statistical difference between them.

Based on ROC analysis (figure 2, 3), both of NLR and SII were significant predictors of DNS in acute CO poisoned patients. For NLR, AUC= 0.711((95% CI 0.581-0.819) (Z=3.141, p=0.0017). The diagnostic criterion using Youden index is the level of >2.69 with a sensitivity of 78.8% (95% CI 61.1-91), specificity of 62.1% (95% CI 42.3-79.3), positive predictive value (PPV) of 70.3% and negative predictive value (NPV) of 72%. While for SII, AUC= 0.741(95% CI 0.614-0.844) (Z=3.866, p=0.0001). The diagnostic criterion using Youden index is the level of >934.93 ($\times 10^3$/ mm^3) with a sensitivity of 63.6% (95% CI 45.1-79.6), specificity of 75.9% (95% CI 56.5-89.7), PPV of 75% and NPV of 64.7%.

Table (1): Demographics, vital signs, arterial blood gases and QTc interval of acute CO poisoned patients at admission and control group.

Variables		Acute CO poisoned patients (n=62)	Control group (n=38)	Test of significance	*p* value
Age (years)	Min-Max Median (IQR)	18-75 24(20-35)	18-70 25(21-32)	$Z_{(MW)}$= -0.210	0.834
Sex: Males **Females**	N (%) N (%)	34 (54.8%) 28 (45.2%)	20 (52.6%) 18 (47.4%)	$X^2_{(df=1)}$= 0.046	0.830
Systolic blood pressure (mmHg)	Min-Max Median (IQR)	80-150 110(100-120)	90-150 120(110-130)	$Z_{(MW)}$= -3.189	0.001*
Diastolic blood pressure (mmHg)	Min-Max Median (IQR)	50-100 65(60-70)	60-100 75(70-80)	$Z_{(MW)}$= -2.988	0.003*
Pulse (beats/min)	Min-Max Median (IQR)	67-153 103(93-120)	75-132 100(89-114)	$Z_{(MW)}$= -1.174	0.240
Respiratory rate (breaths/min)	Min-Max Median (IQR)	12-40 18.5(16-23)	14-26 20(17-22)	$Z_{(MW)}$= -0.545	0.586
Temperature (°C)	Min-Max Median (IQR)	36-38 37.2(37-37.2)	36.5-37.5 37.05(37-37.2)	$Z_{(MW)}$= -1.243	0.214
Oxygen saturation (%)	Min-Max Median (IQR)	40-100 97(89-99)	95-100 98.5(98-99)	$Z_{(MW)}$= -3.341	0.001*
pH	Min-Max Median (IQR)	7.22-7.57 7.42(7.37-7.46)	7.37-7.55 7.43(7.40-7.46)	$Z_{(MW)}$= -1.435	0.151
PCO_2 (mmHg)	Min-Max Median (IQR)	22-330 35.55(31.7-38.9)	25.1-48 34.9(32.1-37.5)	$Z_{(MW)}$= -0.472	0.637
QTc (msec)	Min-Max Median (IQR)	360-516 422.5(404-465)	341-480 408(390-424)	$Z_{(MW)=}$ -2.881	0.004*
QTc interval (Category): **Normal** **Prolonged (>440msec)**	 N (%) N (%)	 36 (58.1%) 26 (41.9%)	 33 (86.8%) 5 (13.2%)	X^2(df=1)= 9.122	0.003*

Min-Max: Minimum-Maximum; IQR: inter-quartile range (25^{th} – 75^{th} percentile); MW: Mann-Whitney test
**: Statistically significant (p<0.05); %: Percent within group; df: : degree of freedom*

Table (2): Hematologic parameters based on peripheral CBC count of acute CO poisoned patients at admission and control group.

Variables		Acute CO poisoned patients (n=62)	Control group (n=38)	$Z_{(MW)}$	*p* value
Hb (%)	Min-Max Median (IQR)	9.1-15.6 12.8(12-13.8)	8.2-15.4 11.9(10.7-12.7)	-3.251	0.001*
Platelets ($x10^3$/ mm^3)	Min-Max Median (IQR)	90-380 242(201-298)	124-398 229(195-278)	-0.856	0.392
White blood cells ($cells/mm^3$)	Min-Max Median (IQR)	48000-19800 8400(6500-11500)	3700-11000 6350(5200-7900)	-3.723	0.000*
Neutrophil ($cells/mm^3$)	Min-Max Median (IQR)	2706-17150 6130(4466-9500)	1730.4-7242 3938(3299-5325)	-4.566	0.000*
Neutrophil (%)	Min-Max Median (IQR)	52-89 74.5(64-82)	40-77 61.05(56-70)	-4.968	0.000*
Absolute lymphocytic count ($cells/mm^3$)	Min-Max Median (IQR)	952-3468 1878(1748-2130)	1097.6-6050 1929(1456-2556)	-0.575	0.565
Lymphocyte (%)	Min-Max Median (IQR)	10-44 23(15-32)	17-55 31.9(25-38)	-4.154	0.000*
Absolute monocytic count ($cells/mm^3$)	Min-Max Median (IQR)	48-1068 225.5(126-380)	80-756 298(240.1- 425)	-2.351	0.019*
Monocyte (%)	Min-Max Median (IQR)	1-9 2.5(1-5)	2-9 5(4-5)	-4.085	0.000*

Table (2): Continued

Variables		Acute CO poisoned patients (n=62)	Control group (n=38)	$Z_{(MW)}$	*p* value
NLR	Min-Max Median (IQR)	1.18-8.91 3.35(1.94-5.3)	0.73-4.53 1.92(1.55-2.84)	-4.417	0.000*
MLR	Min-Max Median (IQR)	0.03-0.69 0.12(0.06-0.22)	0.04-0.32 0.15(0.11-0.22)	-1.631	0.103
PLR	Min-Max Median (IQR)	38.06-363.98 134(108.06-160.56)	37.96-307.94 117.64(96.26-146.48)	-1.520	0.129
SII ($x10^3$/ mm^3)	Min-Max Median (IQR)	168.75-3041.46 869.64(509.13-1327.80)	166.76-1096.99 488.82(330-619.11)	-4.282	0.000*

*Min-Max: Minimum-Maximum; *: Statistically significant (p<0.05); IQR: inter-quartile range (25^{th} – 75^{th} percentile)*
MW: Mann-Whitney test

Table (3): Demographics, admission data, QTc, ECG severity grading, hospital stay and number of needed HBO sessions of DNS-complicated and non-complicated acute CO poisoned patients.

Variables		Acute CO poisoned patients		Test of significance	*p* value
		Non-complicated patients (n=29, 46.8%)	DNS-complicated patients (n=33, 53.2%)		
Age (years)	Min-Max Median (IQR)	18-75 25 (21-35)	18-48 23 (20-35)	$Z_{(MW)=}$-0.955	0.340
Sex: Males **Females**	N (%) N (%)	11(37.93%) 18 (62.07%)	23 (69.7%) 10 (30.3%)	$X^2_{(df=1)}$=6.289	0.012*
Delay (hours)	Min-Max Median (IQR)	0.5-36 3 (1-4)	0.5-36 2 (1-5)	$Z_{(MW)=}$-0.546	0.585
Duration of exposure (hours)	Min-Max Median (IQR)	0.5-12 2 (1-4)	0.5-15 4.5 (2-7)	$Z_{(MW)=}$-2.662	0.008
Initial GCS on admission	Min-Max Median (IQR)	3-15 15 (14-15)	3-15 11 (8-13)	$Z_{(MW)=}$-3.715	0.000*
COHb level (%)	Min-Max Median (IQR)	3-20 10 (6-12)	2-48 12 (8-23)	$Z_{(MW)=}$-2.490	0.013*
QTc (msec)	Min-Max Median (IQR)	364-516 413 (403-465)	360-512 430 (409-467)	$Z_{(MW)=}$-0.783	0.433
QTc interval (Category): **Normal** **Prolonged (>440msec)**	 N (%) N (%)	 18 (62.1%) 11 (37.9%)	 18 (54.5%) 15 (45.5%)	$X^2_{(Y)(df=1)}$= 0.116	0.733
ECG severity grading: **Normal** **Minor** **Moderate** **Severe**	 N (%) N (%) N (%) N (%)	 13(44.8%) 2 (6.9%) 14 (48.3%) 0 (0%)	 8 (24.2%) 4 (12.1%) 19 (57.6%) 2 (6.1%)	$X^2_{(MC)(df=3)}$= 4.375	0.248
Length of hospital stay: **<1day** **1-3days** **> 3days**	 N (%) N (%) N (%)	 16(55.2%) 12(41.4%) 1(3.4%)	 8(24.2) 10(30.3%) 15(45.5%)	$X^2_{(MC)(df=2)}$= 14.902	0.000*
Number of needed HBO sessions	Min-Max Median (IQR)	0-4 1 (0-1)	0-8 4 (1-5)	$Z_{(MW)}$=-3.498	0.000*

Min-Max: Minimum-Maximum; IQR: inter-quartile range (25^{th} – 75^{th} percentile); %: Percent within group;
**: Statistically significant (p<0.05); MC: Monte Carlo correction for Pearson's Chi Square p value;*
MW: Mann-Whitney test; Y: Yates correction for Pearson's Chi Square and its p value; df: degree of freedom

Table (4): Vital signs and arterial blood gases of DNS-complicated and non-complicated acute CO poisoned patients.

Variables		Acute CO poisoned patients		$Z_{(MW)}$	*p* value
		Non-complicated patients (n=29, 46.8%)	DNS-complicated patients (n=33, 53.2%)		
Systolic blood pressure (mmHg)	Min-Max Median (IQR)	100-150 110 (110-120)	80-150 110 (100-120)	-1.278	0.201
Diastolic blood pressure (mmHg)	Min-Max Median (IQR)	50-90 70 (60-70)	50-100 65 (60-75)	-0.298	0.766
Pulse (beats/min)	Min-Max Median (IQR)	69-136 100 (90-120)	67-153 110 (96-125.5)	-0.874	0.382
Respiratory rate (breaths/min)	Min-Max Median (IQR)	12-27 18 (17-23)	12-40 19 (16-22)	-0.241	0.810
Temperature (°C)	Min-Max Median (IQR)	36-37.7 37.2 (37-37.2)	36-38.2 37.2 (37.2-37.5)	-1.358	0.174
Oxygen saturation (%)	Min-Max Median (IQR)	90.1-100 98 (96-99)	40-100 90 (84-98)	-3.424	0.001*
pH	Min-Max Median (IQR)	7.31-7.57 7.43(7.39-7.49)	7.22-7.51 7.42(7.35-7.44)	-1.680	0.093
PCO_2 (mmHg)	Min-Max Median (IQR)	22-49.3 35 (27.8-37.5)	22.8-330 36.2 (33.3-40.2)	-1.778	0.075

*Min-Max: Minimum-Maximum; *: Statistically significant (p<0.05); IQR: inter-quartile range (25^{th} – 75^{th} percentile); MW: Mann-Whitney test*

Table (5): Hematologic parameters based on peripheral CBC count of DNS-complicated and non-complicated acute CO poisoned patients.

Variables		Acute CO poisoned patients		$Z_{(MW)}$	*p* value
		Non-complicated patients (n=29, 46.8%)	DNS-complicated patients (n=33, 53.2%)		
Hb (%)	Min-Max Median (IQR)	9.1-14.8 12.6 (12-13.9)	11-15.6 12.9 (12.3-13.6)	-0.678	0.498
Platelets ($x10^3$/ mm^3)	Min-Max Median (IQR)	90-347 222 (174-298)	156-380 260 (225-295)	-1.898	0.058
White blood cells (cells/mm^3)	Min-Max Median (IQR)	4800-15400 7700 (6200-9200)	4800-19800 9600 (6900-13800)	-2.499	0.012*
Neutrophil (cells/mm^3)	Min-Max Median (IQR)	2706-13491 5440 (3658-6525)	2941-17150 7441 (4841-11250)	-2.758	0.006*
Neutrophil (%)	Min-Max Median (IQR)	52-89 69 (62-79)	57-87 79 (72-83)	-2.768	0.006*
Absolute lymphocytic count (cells/mm^3)	Min-Max Median (IQR)	952-3468 1888 (1748-2314)	998-3024 1875 (1755-2050)	-0.536	0.592
Lymphocyte (%)	Min-Max Median (IQR)	10-44 27 (19-34)	10-37 17 (13-26)	-2.791	0.005*
Absolute monocytic count (cells/mm^3)	Min-Max Median (IQR)	48-656 210 (125-288)	59-1068 276 (138-436)	-1.192	0.233
Monocyte (%)	Min-Max Median (IQR)	1-8 2 (1-5)	1-9 3 (1-4)	-0.050	0.960
NLR	Min-Max Median (IQR)	1.18-8.91 2.51 (1.88-4.21)	1.58-8.47 4.85 (2.83-5.95)	-2.843	0.004*
MLR	Min-Max Median (IQR)	0.03-0.53 0.1 (0.06-0.17)	0.03-0.69 0.15 (0.07-0.26)	-1.448	0.148
PLR	Min-Max Median (IQR)	38.06-216.39 125.98(90.04-147.72)	61.84-363.98 135.48 (123.94-171.2)	-1.686	0.092
SII ($x10^3$/ mm^3)	Min-Max Median (IQR)	168.75-1666.34 730.3 (372.38-934.93)	296.08-3041.46 1122.38 (585-1546.05)	-3.252	0.001*

*Min-Max: Minimum-Maximum; *: Statistically significant (p<0.05); IQR: inter-quartile range (25^{th} – 75^{th} percentile) MW: Mann-Whitney test*

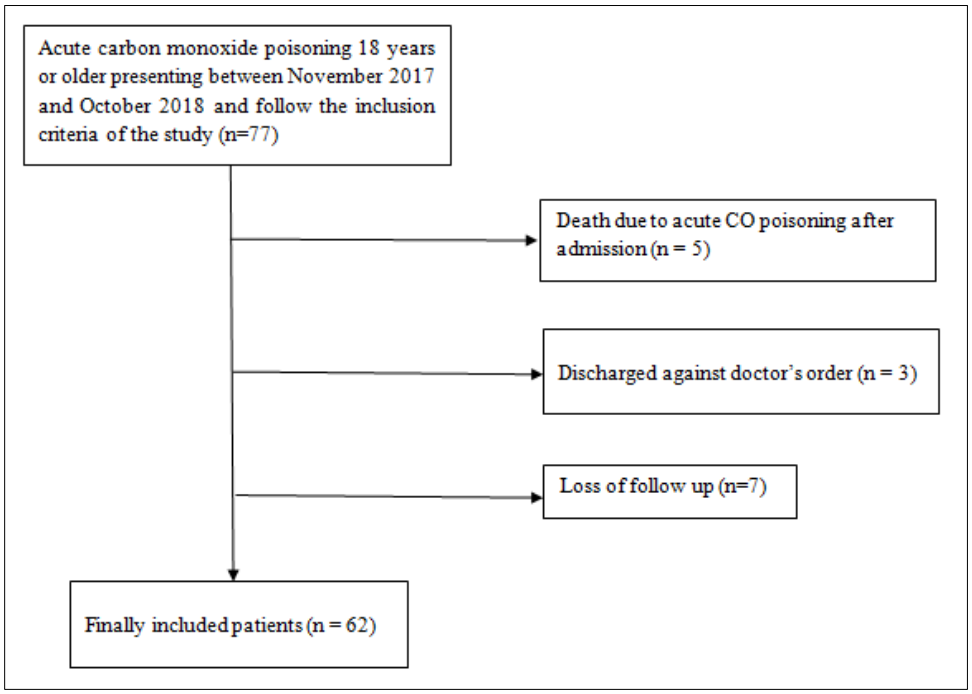

Figure (1): Algorithm for patient selection

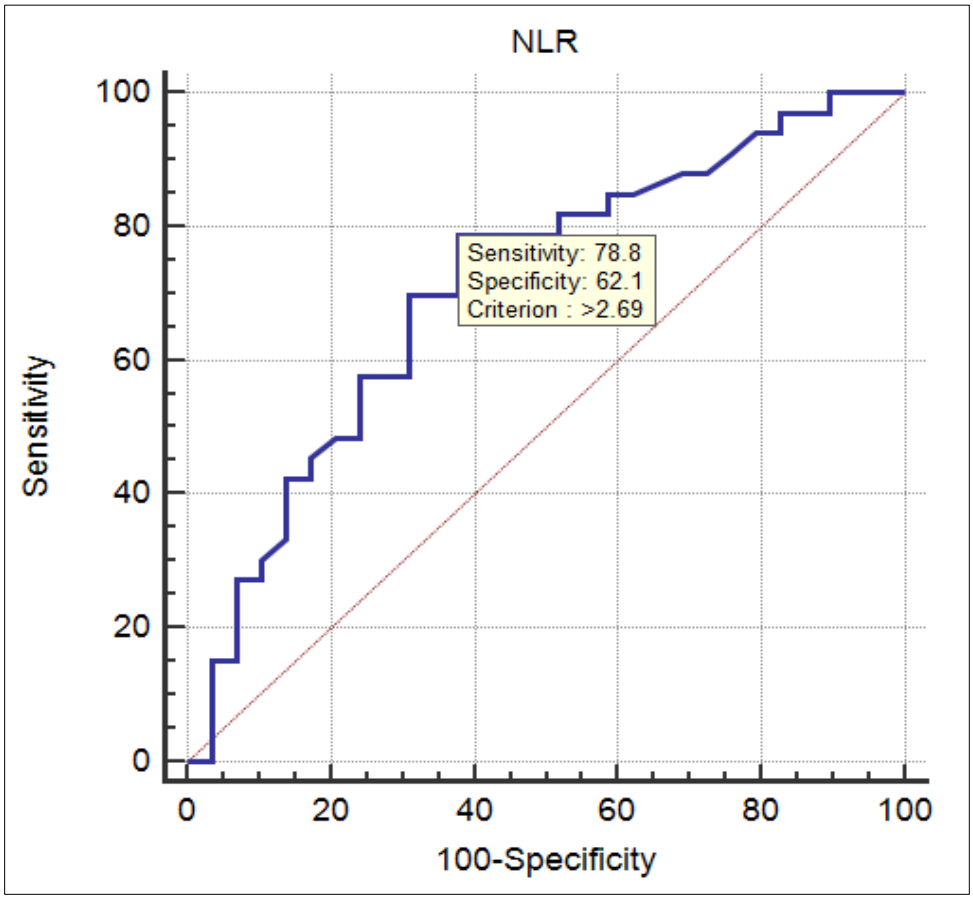

Figure (2): Area under ROC curve of NLR for discrimination of DNS in acute CO poisoned patients.

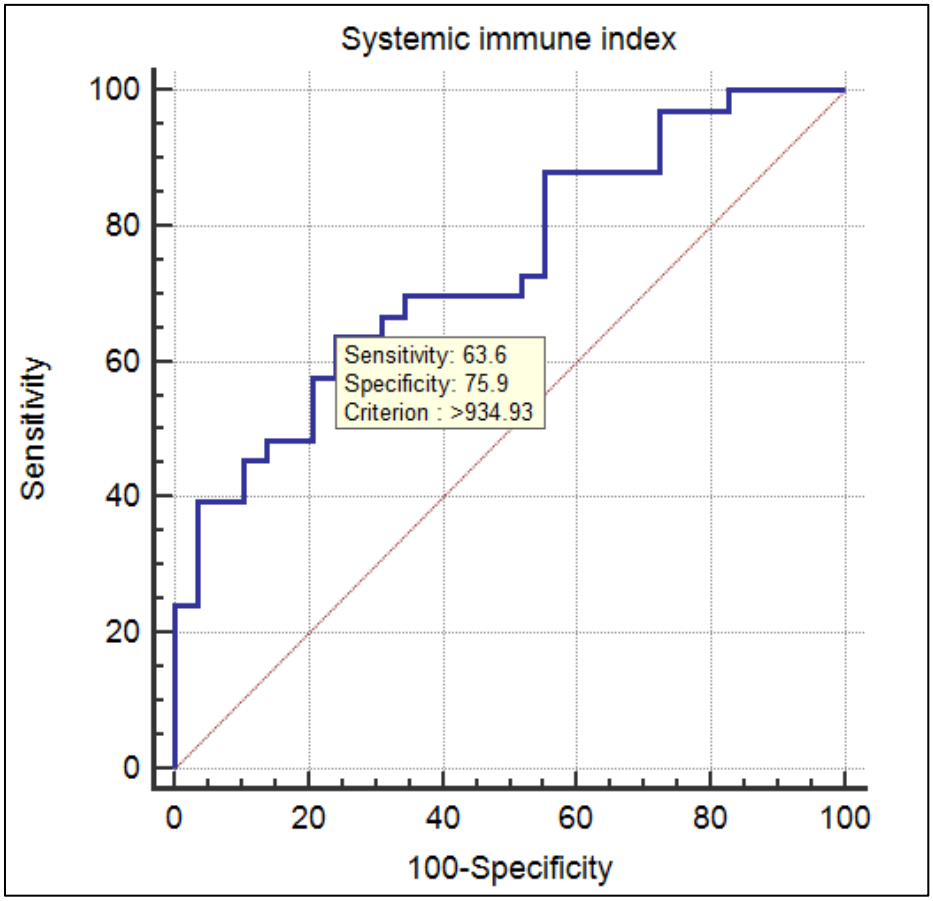

Figure (3): Area under ROC curve of SII for discrimination of DNS in acute CO poisoned patients.

Discussion

Acute CO poisoning is found as frequent emergency medical condition worldwide that have high morbidity and mortality *(Sönmez et al., 2018)*. After CO intoxication, recurrent transient DNS may occur with alternating periods of exacerbation and remission. Moreover, DNS causes diffuse white matter or gray matter injury and may be reversible *(Beppu et al., 2011; Kuroda et al., 2015)*. So, early prediction of DNS is very important in toxicological emergencies.

Nowadays, new hematological ratios based on peripheral CBC count are available as new inflammatory prognostic marker. Based on this, this study aimed to investigate whether hematological parameters based on peripheral CBC counts are associated with occurrence of DNS after acute CO poisoning and which parameters could predict them.

Concerning vital signs, this study showed that median of blood pressure (both systolic and diastolic) and oxygen saturation were significantly lower while QTc interval was significantly longer when comparing the included acute CO poisoned patients with control group. These were more or less in agreement with many previous observations in majority of centers in Egypt and across the world *(Ismail et al., 2013; Abass et al., 2017; Liao et al., 2019b)*. The possible mechanism of QTc interval prolongation in CO poisoning is disruption of repolarization caused by late inward Na+ current resulting in action potential prolongation *(Dallas et al., 2012)*.

Regarding hematological parameters, total WBC count, absolute neutrophil count and percentage were significantly higher in acute CO poisoned patients compared to control group. This was in accordance with *Karabacak et al. (2015)* who found higher neutrophil count at presentation in severe CO poisoned patients compared to control group. This could be attributed to degranulation of intravascular neutrophils after acute CO poisoning, which induces generation of reactive oxygen species and catalyzes lipid peroxidation in human *(Thom et al., 2006b)*. Moreover, neutrophils activation promotes the synthesis of inflammatory cytokines so; they are potential biomarkers for inflammation *(Huang et al., 2018b)*.

On the other hand, lymphocytes percentage, absolute monocytic count and percentage were significantly lower in acute CO poisoned patients compared to control group in this study. This could be attributed to physiological stress leading to corticosteroids and catecholamine release after the activation of neurohormonal system to increase pro-inflammatory cytokine levels. These corticosteroids and catecholamine release in turn

mediates lymphocyte apoptosis and lymphocytopenia in peripheral blood *(Moon et al., 2019b)*.

Moreover, this study found that median values of NLR and SII were significantly higher in acute CO poisoned patients when compared to control group. Previous researches have detected NLR and SII as simple powerful markers of ongoing nonspecific inflammation by combining of independent parameters (neutrophils, lymphocytes, platelets) *(Hu et al., 2014; Karabacak et al., 2015)*. This confirmed ongoing inflammation induced by CO poisoning that may play important role in the pathogenesis of CO complications included DNS.

The incidence of DNS in the studied acute CO patients was 53.2% which was more or less comparable with previous reports which indicted variable incidence of DNS in acute CO poisoned patients *(Ku et al., 2010; Kudo et al., 2014; Du et al., 2019)*. These variations in the incidence of DNS could be attributed to various factors e.g. the studied patients' age, duration of CO exposure, delay time before hospitalization and CO concentration.

This neurological sequalae could be attributed to mitochondrial oxidative stress in the neuronal cells by free radicals generation, white matter demyelination by CO immune response, post ischemic reperfusion injury, apoptosis and inflammation *(Khot et al., 2007; Sönmez et al., 2018)*. Moreover, abnormal inflammatory reaction of acute CO poisoning may be long lasting after the initial stage of poisoning *(Lippi et al., 2012; Rose et al., 2017)*.

Regarding demographics, admission data, vital signs, QTc, ECG severity grading, COHb level, hospital stay and number of needed HBO sessions, DNS-complicated patients had significantly lower initial GCS and oxygen saturation and higher COHb level on admission, length of hospital stay and number of needed HBO sessions than non-complicated ones.

Initial low GCS at admission was reported as early predictor of DNS in acute CO poisoning in many previous studies *(Ku et al., 2010; Kudo et al., 2014; Du et al., 2019)*. This may be due to CO induced neuronal injury by its hypoxic and inflammatory effect. This was in accordance with *Cha et al. (2018)* who concluded that patients who have disturbed consciousness in the acute phase of CO poisoning, can be easily complicated with DNS in the subacute or chronic phase.

Regarding COHB levels at admission, *Cervellin et al. (2014) and Hampson (2018)* demonstrated that elevated COHb levels are only exposure indicator and does not correlate with acute CO poisoning severity or neurological deficits. Furthermore, length of hospital stay was longer in the DNS-complicated patients; this was in accordance with *Kudo et al. (2014)* because these patients usually needed longer time to recover from the acute phase of CO poisoning.

Regarding treatment with HBO sessions, this study showed that DNS-complicated patients received more HBO sessions than non-complicated one. This means that the risk for DNS in patients who received HBO sessions was higher than that in patients who did not. This was in line with results of many previous studied assumed that the supplemental benefit of HBO therapy for reducing DNS has been a matter of debates *(Annane et al., 2011; Hampson et al., 2012; Huang et al., 2017)*.

This could be explained by *Huang et al. (2018a)*, who stated that HBO sessions had more prominent life-saving effect than their possible DNS-reducing effect. This may be attributed to that HBO sessions were the main line of treatment in acute CO intoxicated patients who were also at higher risks for DNS but these patients have passed away before completed the needed number of sessions due to different treatment protocols *(Hosseininejad et al., 2018)*. This confirms the fact that there must be easy, cheap and widely available laboratory marker of ongoing systemic inflammation in acute CO intoxicated patients to decide whether patient is in need for further HBO sessions or not before leaving hospital.

No statistical difference was detected between DNS-complicated and non-complicated patients regarding QTc interval and ECG severity grading in this study. On the other hand, *Liao et al. (2018)* found QTc interval significantly prolonged in DNS-complicated patients. These confounded results are reasonable because CO is eliminated through pulmonary circulation within about 5 hours under room air breathing *(Chang et al., 2017)*. In our study, the delay time was ranged from 0.5 to 36 hours which could lead to pulmonary decontamination of CO.

This study had investigated different hematological parameters based on peripheral CBC counts (NLR; MLR; PLR and SII) as a predictor of DNS in acute CO poisoned patients and which parameters might significantly improve the predictive accuracy for DNS. Median values of NLR and SII were significantly higher in DNS-complicated patient than non-complicated ones in this study. Several studies demonstrated the relevance between ongoing inflammation and DNS after CO poisoning *(Thom et al., 2004;Thom et al., 2006a;Thom et al., 2006b)*.

During the inflammatory process, leucocytes and platelets circulating in the blood act in conjunction with endothelial cells. Each subtype of leucocyte has a different role in the inflammatory and immunological process and contributes differently to the pathophysiology *(Moon et al., 2019a)*. Platelets also contribute by releasing numerous inflammatory mediators that modify leucocyte and endothelial responses to a range of inflammatory stimuli *(Thomas and Storey, 2015)*.

Based on ROC analysis, both NLR and SII were significant predictors of DNS in CO poisoned patients (AUCs: 0.711 and 0.741 respectively). This was in accordance with *Moon et al. (2019a)* who detected that AUCs of NLR and SII of CO poisoned patients over the first 12 hours after presentation were >0.70. The acceptable performance of these comprehensive inflammatory ratios may be attributed to that these ratios are indicating both the pro-inflammatory and immunosuppression status by integrating two or three

types of circulating immune-inflammatory cells, such as neutrophils, monocytes, lymphocytes and platelets *(Moon et al., 2019b)*. Neutrophils are markers of ongoing nonspecific inflammation while lymphocytes are markers of the regulatory pathway *(Karabacak et al., 2015)*.

Moreover, these simple and cheap ratios can be measured easily at presentation by routine CBC count testing. They can be used by emergency physicians as early predictors of DNS in acute CO poisoned patients that are considered the most serious complication after apparent recovery from acute CO poisoning. This will help emergency physicians to decide more sufficient HBO sessions for those patients to decrease neurological insult and to prevent early discharge of patients from hospital before enough treatment.

Conclusion

This study demonstrated that NLR and SII ratios based on peripheral CBC count may be helpful predictors of DNS after acute CO poisoning. Both can be used as simple measureable promising parameters predicting DNS after acute CO poisoning.

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العوامل الدموية كمتنبؤات مبكرة للعواقب العصبية المتأخرة في التسمم الحاد بأول أكسيد الكربون

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الملخص العربى

المقدمة:

المقدمة: تعد العواقب العصبية المتأخرة (DNS) المرض الأكثر شيوعًا بعد التسمم الحاد بأول أكسيد الكربون. لذلك ، فإن الوقاية والتنبؤ المبكر هما الهدف الرئيسي للعلاج.**الهدف من الدراسة:** تهدف هذه الدراسة إلى فحص عدد من العوامل على أساس عدد خلايا الدم الكاملة (CBC) كمتنبئات للعواقب العصبية المتأخرة بعد التسمم الحاد بأول أكسيد الكربون. **طريقة البحث:** شملت الدراسة ٦٧ من مرضى التسمم الحاد بأول أكسيد الكربون و ٣٨ متطوعًا صحيًا. عند دخول المستشفى، تم أخذ التاريخ المرضي والفحص السريري والفحوصات المخبرية الروتينية. تم قياس النسب على أساس عدد خلايا الدم الكاملة، تمت متابعة المرضى لمدة ٦ أشهر لإشارات العواقب العصبية المتأخرة. **النتائج:** قد أظهرت النتائج أن إجمالي عدد خلايا الدم البيضاء (WBC) العد المطلق لخلايا النيوتروفيل والنسبة المئوية والقيم المتوسطة لنسبة خلايا النيوتروفيل للخلايا اللمفاوية (NLR) ومؤشر الالتهاب المناعي العام ((SII) أعلى بشكل ملحوظ بينما كانت نسبة الخلايا الليمفاوية والعد المطلق لخلايا المونوسايت ونسبتها المئوية أقل بكثير في مرضى التسمم الحاد بأول أكسيد الكربون مقارنة بالمجموعة الضابطة. كان لدى المرضى الذين يعانون من تعقيدات العواقب العصبية المتأخرة عددًا أقل بكثير من إجمالي عدد خلايا الدم البيضاء والعد المطلق لخلايا النيوتروفيل ونسبتها المئوية وقيم متوسط أعلى بكثير من نسبة خلايا النيوتروفيل للخلايا اللمفاوية (NLR) ومؤشر الالتهاب المناعي العام (SII) أكثر من نسب المرضى الذين لم تظهر عليهم العواقب العصبية المتأخرة. واستنادا إلى تحليل منحنى (ROC) ،كانت نسبة خلايا النيوتروفيل للخلايا اللمفاوية (NLR) ومؤشر الالتهاب المناعي العام (SII) متنبئين مهمين للعواقب العصبية المتأخرة (DNS).**الخلاصة:** تم استنتاج أن نسبة خلايا النيوتروفيل للخلايا اللمفاوية (NLR) و مؤشر الالتهاب المناعي العام (SII) قد تكون عوامل مساعدة في التنبؤ بالعواقب العصبية المتأخرة (DNS) بعد التسمم الحاد بأول أكسيد الكربون.

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