Circulation: Heart Failure

ORIGINAL ARTICLE

Increased Risk of Congestive Heart Failure Following Carbon Monoxide Poisoning

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BACKGROUND: Carbon monoxide poisoning (COP) is an important public health issue around the world. It may increase the risk of myocardial injury, but the association between COP and congestive heart failure (CHF) remains unclear. We conducted a study incorporating data from epidemiological and animal studies to clarify this issue.

METHODS: Using the National Health Insurance Database of Taiwan, we identified patients with COP diagnosed between 1999 and 2012 and compared them with patients without COP (non-COP cohort) matched by age and the index date at a 1:3 ratio. The comparison for the risk of CHF between the COP and non-COP cohorts was made using Cox proportional hazards regression. We also established a rat model to evaluate cardiac function using echocardiography and studied the pathological changes following COP.

RESULTS: The 20 942 patients in the COP cohort had a higher risk for CHF than the 62 826 members in the non-COP cohort after adjusting for sex and underlying comorbidities (adjusted hazard ratio, 2.01 [95% CI, 1.74–2.32]). The increased risk of CHF persisted even after 2 years of follow-up (adjusted hazard ratio, 1.85 [95% CI, 1.55–2.21]). In the animal model, COP led to a decreased left ventricular ejection fraction on echocardiography and damage to cardiac cells with remarkable fibrotic changes.

CONCLUSIONS: Our epidemiological data showed an increased risk of CHF was associated with COP, which was supported by the animal study. We suggest close follow-up of cardiac function for patients with COP to facilitate early intervention and further studies to identify other long-term effects that have not been reported in the literature.

Key Words: animals ■ carbon monoxide poisoning ■ echocardiography ■ epidemiology ■ heart failure

Carbon monoxide poisoning (COP) is an important public health issue worldwide, accounting for 50 000 emergency department visits and 1300 deaths in the US annually.[1,2] In Asia, COP by burning charcoal is a common method for suicide.[3] In Taiwan, about 20% to 30% of patients with COP had underlying mental disorders,[4,5] and a hospital-based study reported that about 90% of their COP cases were due to suicide attempts.[4]

Carbon monoxide (CO) is an odorless, colorless, and tasteless gas produced from incomplete combustion of carbon compounds, including engine exhaust, fire, charcoal, and furnaces.[1] The major mechanisms of its toxic effect are hypoxic injury due to the 250-fold higher affinity for hemoglobin than oxygen, free radical generation, mitochondrial inhibition, and inflammation.[1,6] The brain and heart are the most common organs injured by COP because they have the highest oxygen demands.[1,6–8] A nationwide study in Taiwan showed a 45% increase in the risk for myocardial injury in patients with COP.[9]

Despite the increased myocardial injuries associated with COP, it is unclear whether COP may also lead to congestive heart failure (CHF). A study found that over

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WHAT IS NEW?

- Our epidemiological study showed that patients with carbon monoxide poisoning had a ≈2-fold higher risk of developing congestive heart failure after adjusting for sex and underlying comorbidities.
- The increase in the risk of congestive heart failure was highest in the first month of follow-up, nearly 13-folds, and persisted even after 2 years.
- In our animal model, carbon monoxide poisoning led to a decreased left ventricular function and damages to cardiac cells with remarkable fibrotic changes, which are comparable to the finding of increased congestive heart failure observed in humans.

WHAT ARE THE CLINICAL IMPLICATIONS?

- Close follow-up of cardiac function after patients encountered carbon monoxide poisoning is recommended to facilitate early intervention for prevention of congestive heart failure, especially in the first month after the poisoning.
- The monitoring of cardiac function in patients with carbon monoxide poisoning should last for >2 years.
- The monitoring of cardiac function in patients with carbon monoxide poisoning is more important in patients with hypertension, diabetes, hyperlipidemia, obesity, and low income.

Nonstandard Abbreviations and Acronyms

AHR	adjusted hazard ratio
CHF	congestive heart failure
COP	carbon monoxide poisoning
ICD-9-CM	International Classification of Diseases, Ninth Revision, Clinical Modification
LVEF	left ventricular ejection fraction
NHIRD	National Health Insurance Research Database

half of the patients with COP had a decreased left ventricular ejection fraction (LVEF<45%), but their cardiac function recovered at the follow-up echocardiography 24 hours later.[8] Another study showed that patients with COP did not have an increased risk for CHF (adjusted hazard ratio [AHR]=1.07 [95% CI, 0.77–1.46]).[10] However, both studies included hospitalized patients only, but a substantial proportion of patients with COP do not require hospitalization. Therefore, we conducted an epidemiological study that included all patients with COP, regardless of hospitalization or not, to delineate the association between COP and CHF. We also conducted an animal study to evaluate cardiac dysfunction using echocardiography and cardiac damage using histopathology to correlate with findings in the epidemiological study.

METHODS

Epidemiological Study

We used the National Health Insurance Research Database (NHIRD) of Taiwan to conduct a nationwide population-based cohort study. Because nearly 100% of Taiwan's population was enrolled, the NHIRD has become a useful source for generating evidence to support making clinical decisions and health policy.[11]

Study and Comparison Cohorts

We identified patients with COP from the NHIRD by the *International Classification of Diseases, Ninth Revision*, *Clinical Modification* (*ICD-9-CM*) codes 986, E868, E952, and E982 either at the time of hospitalization or emergency department care between 1999 and 2012 as the study cohort (COP cohort).[12,13] From the NHIRD, a comparison cohort of enrollees without COP (non-COP cohort) was selected by matching age and index date (the date when COP was diagnosed) at a 3:1 ratio with the COP cohort. Members of the non-COP were selected from those who visited the emergency department or were hospitalized on the index date of the matched patient with COP but were not diagnosed with COP. All patients who had a history of acute pericarditis (*ICD-9-CM*: 420), acute and subacute endocarditis (*ICD-9-CM*: 421), acute myocarditis (*ICD-9-CM*: 422), other diseases of pericardium (*ICD-9-CM*: 423), other diseases of endocardium (*ICD-9-CM*: 424), cardiomyopathy (*ICD-9-CM*: 425), conduction disorders (*ICD-9-CM*: 426), cardiac dysrhythmias (*ICD-9-CM*: 427), heart failure (*ICD-9-CM*: 428), and ill-defined descriptions and complications of heart disease (*ICD-9-CM*: 429) in at least one hospitalization or 3 ambulatory care visits before the index date were excluded.

In Taiwan, COP is generally diagnosed based on the following criteria: (1) documented CO exposure, including elevated carboxyhemoglobin levels or ambient CO concentrations and (2) any related symptoms (loss of consciousness, confusion, headache, dizziness, nausea, vomiting, malaise, fatigue, forgetfulness, visual disturbances, and cardiac ischemia) or metabolic acidosis (a blood lactate level >2.5 mmol/L or a calculated base excess level <−2.0 mmol/L).[13] If the patient had a carboxyhemoglobin level <10%, COP was diagnosed when COP was the only probable diagnosis based on clinical manifestations.[13]

Definition of Variables

Age was classified into the subgroups of ≤34, 35 to 49, 50 to 64, and ≥65 years old.[9] The following underlying comorbidities in both cohorts were included in the analyses: (1) hypertension (*ICD-9-CM* 401–405); (2) diabetes: *ICD-9-CM* 250; (3) hyperlipidemia: *ICD-9-CM* 272; (4) ischemic heart disease: *ICD-9-CM* 410 to 414; (5) thyroid disease: *ICD-9-CM* 193, 240 to 246; (6) alcoholism: *ICD-9-CM* 291, 303, 305.0, 357.5, 425.5, 535.3, 571.0 to 571.3, V113; (7) chronic obstructive pulmonary disease: *ICD-9-CM* 496; (8) malignancy: *ICD-9-CM* 140–208; (9) drug abuse: *ICD-9-CM* 303–305; (10) HIV infection: *ICD-9-CM* 042, 079.53, V08; (11) obesity: *ICD-9-CM* 278; and (12) depression: *ICD-9-CM* 296.2, 296.3, 296.5, 296.82, 300.4, 309.0, 309.1, 311. Patients who had these diagnostic codes in at least one hospitalization or 3 ambulatory care claims before the index date were defined as having the comorbidity. The above-mentioned comorbidities are risk factors for CHF,[14] and

thus, are potential confounding factors. Acute respiratory failure was identified by *ICD-9-CM* diagnostic codes (518.81 or 518.84) or management codes (311, 9390, 9391, 960, 9601 to 9605) in the index visit for COP. We also included monthly income as an indicator of socioeconomic status.

Outcome Measurement

We compared the risk of CHF (*ICD-9-CM* 428 in at least one hospitalization or 3 ambulatory care claims) between the COP and non-COP cohorts by following up until 2013.

Animal Study

We obtained 11 adult male Wistar rats (8–10 weeks old, weighing 380–420 g) from BioLASCO Taiwan Co, Ltd (Taipei, Taiwan). We used only male rats because females have menstrual cycles, which may influence some physiological parameters.[15] The rats were housed in groups of 4 per cage in a temperature- and humidity-controlled animal facility with a 12-hour light-dark cycle (08:00–20:00). Food and water were available ad libitum.

COP Rat Model

Rats were exposed to 1000 ppm CO in the air for 20 minutes followed by 3000 ppm CO for another 80 minutes in 98 liters (38.1 cm × 50.8 cm × 50.8 cm). Plexiglas chamber as described previously.[16] In the human, carboxyhemoglobin ≥40% was found to be a threshold of loss of consciousness, which indicated acute severe COP.[17] We had tried the protocols repeatedly to find the best one to achieve the level of acute severe COP (carboxyhemoglobin ≥40%). Rats were then removed from the chamber to breathe fresh room air and regain consciousness.[16] During the whole experiment, a constant flow of CO was set, and the concentration of CO was monitored by a CO analyzer (TPI-708 CO analyzer, Songdo-Dong, Yeonsu-Gu Incheon, Korea). We drew 0.3 mL of whole blood from the tail artery for the carboxyhemoglobin assay after proper anesthesia with isoflurane (1% in O_2) inhalation. A blood gas analyzer (Nova Biomedical, Waltham, MA) was used for carboxyhemoglobin determination. Rats with coma and carboxyhemoglobin ≥40% were regarded as successful in this model. Any rats with near death were removed and excluded from the experimental statistics. The echocardiography was performed before COP (pre-COP) and 1, 7, 14, 21, and 28 days after the induction of COP (post-COP). Rats were euthanized following the final echocardiography examination (day 28 post-COP) with an intraperitoneal injection of 120 mg/kg sodium pentobarbital, perfusion with 4% paraformaldehyde in PBS intracardially, and their hearts were removed and preserved in 10% formalin.

Conventional 2-Dimensional Echocardiography Analysis for Rats

To evaluate the effects of CO on hemodynamics and cardiac contractility, we performed echocardiography on rats. The test was done on rats under 1% isoflurane anesthesia, before and after COP using a 2-dimensional, harmonics, color, M-mode, and pulsed-wave Doppler Echo (GE Vivid S6 Cardiovascular Ultrasound System; GE Healthcare, Chicago, IL) equipped with a 12S-RS sector (Catalog no. H44901AB, GE Healthcare, Chicago, IL) centered at 5.0 to 11.0 MHz (13×18 mm footprint, 5.0–11.0 MHz scanner frequency ranges, 90° field of view, 12 cm depth of field). Measurements including LV interventricular septum thickness in diastole, LV internal dimension in diastole, LV internal dimension in systole, LV posterior wall thickness in diastole, LV end-diastolic volume, LV end-systolic volume, LVEF, LV fractional shortening, LV stroke volume, LV mass in diastole, heart rate, and LV cardiac output were recorded. These measurements were averaged over 3 cardiac cycles in 11 rats. We also performed echocardiography on 11 healthy rats without COP as controls to demonstrate the normal changes and variability over time.

Histopathologic Examination

On day 28 post-COP, rats were euthanized, and their hearts were processed for paraffin embedding. Five-micrometer-thick sagittal sections were cut serially and stained with hematoxylin and eosin. We used an optical microscope (Carl Zeiss, Jena, Germany) to identify histopathologic changes. The myocyte damage was graded as follows: 0, no abnormal finding; 1, isolated focus of early ischemic changes (edema, waviness of fibers, or loss of cross striations); 2, multifocal early ischemic changes; 3, single focus of moderate coagulative necrosis (shrunken eosinophilic cytoplasm, pyknosis, or loss of nuclei; 4, multifocal moderate coagulative necrosis; and 5, multifocal severe necrosis with total loss of tissue architecture).[18]The extent of the affected area was graded as follows: 0, no lesion; 1, presence of a few small lesions, not exceeding 0.25 mm^2 in size; 2, presence of multiple small lesions or a few moderately sized lesions, not exceeding 6.25 mm^2; and 3, presence of multiple moderately sized lesions or more, larger lesions.[19,20]

Heart samples were processed, embedded in paraffin, sectioned, and stained with Masson trichrome stain (Sigma-Aldrich, United States) to confirm fibrosis evidenced by fiber extension and collagen accumulation. All protocols and reagents were used as per the manufacturer's instructions. In brief, the sections were soaked in Weigert iron hematoxylin working solution for 15 minutes and then in Biebrich scarlet-acid fuchsin solution for another 15 minutes. Then, we soaked the sections in aniline blue to detect the area of fibrosis in the heart tissue, with blue areas indicating fibrotic staining. We captured images using a digital camera linked to a computer running Axioscope Version 4 (Carl Zeiss, Jena, Germany). To quantify the Masson trichrome-stained images, we randomly selected 5 fields for each sample and used Image-Pro Plus software (Media Cybernetics, Bethesda, MD) to calculate the percentage of fibrosis area in the interstitial and vascular region.[21]

Cardiac Toxic Marker Troponin-I Measurement

All rats were drawn 0.5 mL of blood from the tail vein for the measurement of baseline level of troponin-I using ARCHITECT i2000SR immunoassay analyzer (Abbott Laboratories, Lake Forest, IL). After COP induction, rats were also drawn 0.5 mL of blood from the tail vein on days 1, 7, 14, 21, and 28 to measure the troponin-I levels.

Ethical Statements

We conducted this study according to the Declaration of Helsinki and after obtaining approval from the institutional

review board at the study hospital. As the epidemiological study was an analysis of an anonymous database, the institutional review board waived the need for patients' informed consent. The waiver did not affect the welfare of the patients. All animal experiments were approved by the Institutional Animal Care and Use Committee of the study hospital (no. 108041101) and performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and the number of animals euthanized.

Statistical Methods

In the epidemiological study, we used independent *t* tests for continuous variables and χ^2 tests for categorical variables to evaluate the differences in demographic characteristics and underlying comorbidities between the 2 cohorts. Cox proportional hazards regression as well as the Kaplan-Meier method with a log-rank test were performed to compare the risk of CHF between the 2 cohorts. Multivariate analyses were performed following univariate analyses to investigate independent predictors for CHF. We also performed stratified analyses according to age subgroup, sex, and underlying comorbidities with propensity score matching to account for the possible imbalances of potential confounding factors that were not included in the models. The propensity score was calculated from a multivariable logistic regression model with age, sex, comorbidities, and monthly income as covariates. An SAS matching macro %OneToManyMTCH with a greedy matching algorithm was used to match the propensity score.[22] Because death is a competing risk of the outcome evaluated in this study, we also performed a competing risk survival analysis to compare with results from Cox proportional hazards regression. All patients were right-censored to the new-onset CHF, the date of death, or the end of follow-up date.

In the animal study, data were presented as the mean±SD and were analyzed using independent *t* tests for comparisons between 2 groups. In the comparisons of echocardiography and troponin-I between pre-COP and post-COP rats, we used 1-way ANOVA with repeated measures followed by paired comparisons with the baseline value when the ANOVA indicated significant changes. SAS 9.4 for Windows (SAS Institute, Cary, NC) was used for all analyses. The significance level was set at 0.05 (2 tailed).

Anonymized data from the NHIRD have been made publicly available upon approval of application to the Ministry of Health and Welfare, Taiwan, R.O.C. at the researcher's cost. The animal experiment data that support the findings of this study are available from the corresponding author upon reasonable request.

RESULTS

In the epidemiological study, we identified 20 942 patients with COP and 62 826 patients without COP (Table 1). The mean age was 35.0 years in both cohorts because of matching. The proportion of females was 50% in COP cohort and 51% in the non-COP cohort. Compared with the non-COP cohort, the COP cohort had higher prevalence rates of hypertension, diabetes, hyperlipidemia, alcoholism, chronic obstructive pulmonary disease, malignancy, drug abuse, HIV infection, and depression but lower prevalence rates of ischemic heart disease, thyroid disease, and obesity. The distribution of patient sources in the COP cohort was similar to that in the non-COP cohort: 21.0% versus 23.3% from outpatient clinics, 72.2% versus 70.4% from emergency departments, and 6.8% versus 6.3% from hospitalization.

Table 1. Comparison of Demographic Characteristics and Underlying Comorbidities Between COP and Non-COP Cohorts

Variable	COP cohort, n=20 942	Non-COP cohort, n=62 826	*P* value
Age, y	35.0±14.3	35.0±14.3	0.984
Age, y			>0.999
≤34	11 060 (52.8)	33 182 (52.8)	
35–49	6743 (32.2)	20 229 (32.2)	
50–64	2438 (11.6)	7312 (11.6)	
≥65	701 (3.4)	2103 (3.4)	
Sex			<0.001
Female	10 359 (49.5)	32 196 (51.3)	
Male	10 583 (50.5)	30 629 (48.8)	
Underlying comorbidity			
Hypertension	1704 (8.1)	4787 (7.6)	0.015
Diabetes	1003 (4.8)	2373 (3.8)	<0.001
Hyperlipidemia	1300 (6.2)	3618 (5.8)	0.017
Ischemic heart disease	514 (2.5)	2965 (4.7)	<0.001
Thyroid disease	598 (2.9)	3199 (5.1)	<0.001
Alcoholism	687 (3.3)	513 (0.8)	<0.001
Chronic obstructive pulmonary disease	149 (0.7)	322 (0.5)	<0.001
Malignancy	431 (2.1)	1004 (1.6)	<0.001
Drug abuse	883 (4.2)	556 (0.9)	<0.001
HIV infection	55 (0.3)	41 (0.1)	<0.001
Obesity	108 (0.5)	581 (0.9)	<0.001
Depression	2984 (14.3)	3410 (5.4)	<0.001
Monthly income (NTD)			
<19 999	15 014 (71.7)	38 754 (61.7)	<0.001
20 000–39 999	4729 (22.6)	17 802 (28.3)	
≥40 000	1199 (5.7)	6270 (10.0)	

Data are expressed as mean±SD or n (%). COP indicates carbon monoxide poisoning; and NTD, New Taiwan Dollars.

The Cox proportional hazards regression showed that the COP cohort had a higher risk for CHF after adjusting for sex, hypertension, diabetes, hyperlipidemia, ischemic heart disease, thyroid disease, alcoholism, chronic obstructive pulmonary disease, malignancy, drug abuse, obesity, depression, monthly income, and index year (AHR, 2.01 [95% CI, 1.74–2.32]; Table 2). In stratified analyses, the increase in the risk was highest in the first month of follow-up (AHR, 12.70 [95% CI, 5.73–28.12]) and persisted even after 2 years (AHR, 1.85 [95% CI, 1.55–2.21]). Patients with COP had

Table 2. Comparison of the Risk for Congestive Heart Failure Between COP and Non-COP Cohorts by Cox Proportional Hazards Regression

Variable	COP cohort, n=20 942			Non-COP cohort (reference), n=62 826			Crude HR (95% CI)	AHR (95% CI)*
	Case (%)	Person-year	Rate†	Case (%)	Person-year	Rate†		
Overall analysis	311 (1.5)	105 151.7	3.0	604 (1.0)	340 079.6	1.8	1.66 (1.45–1.91)	2.01 (1.74–2.32)
Stratified analysis								
Age, y								
≤34	46 (0.4)	60 110.4	0.8	57 (0.2)	188 522.5	0.3	2.53 (1.71–3.73)	2.43 (1.62–3.64)
35–49	99 (1.5)	32 833.5	3.0	158 (0.8)	108 646.0	1.5	2.08 (1.62–2.68)	2.53 (1.93–3.31)
50–64	83 (3.4)	9642.4	8.6	166 (1.7)	33 363.5	5.0	1.73 (1.33–2.25)	2.00 (1.52–2.62)
≥65	83 (11.8)	2565.3	32.4	223 (10.6)	9547.8	23.4	1.39 (1.08–1.79)	1.61 (1.24–2.09)
Sex								
Female	139 (1.3)	53 901.1	2.6	300 (0.9)	175 501.8	1.7	1.51 (1.23–1.85)	2.07 (1.68–2.55)
Male	172 (1.6)	51 250.5	3.4	304 (1.0)	164 577.8	1.9	1.81 (1.50–2.18)	2.02 (1.66–2.45)
Underlying comorbidity								
Hypertension	101 (5.9)	6267.0	16.1	242 (5.1)	20 437.9	11.8	1.37 (1.08–1.72)	1.50 (1.19–1.91)
Diabetes	58 (5.8)	3453.4	16.8	133 (5.6)	9793.9	13.6	1.24 (0.91–1.69)	1.52 (1.11–2.09)
Hyperlipidemia	53 (4.1)	4629.6	11.5	109 (3.0)	14 456.1	7.5	1.52 (1.10–2.12)	1.74 (1.24–2.44)
Ischemic heart disease	38 (7.4)	1902.5	20.0	304 (10.3)	17 264.9	17.6	1.11 (0.79–1.56)	0.84 (0.59–1.19)
Thyroid disease	5 (0.8)	2429.3	2.1	63 (2.0)	17 948.9	3.5	0.61 (0.24–1.51)	0.67 (0.26–1.72)
Alcoholism	9 (1.3)	2472.5	3.6	8 (1.6)	2068.6	3.9	0.98 (0.38–2.54)	0.92 (0.32–2.67)
Chronic obstructive pulmonary disease	16 (10.7)	507.5	31.5	32 (10.0)	1343.1	23.8	1.31 (0.70–2.42)	1.31 (0.66–2.58)
Malignancy	14 (3.3)	1308.7	10.7	30 (3.0)	4087.0	7.3	1.45 (0.77–2.74)	1.72 (0.86–3.44)
Drug abuse	9 (1.0)	3024.6	3.0	9 (1.6)	1976.8	4.6	0.65 (0.26–1.64)	0.97 (0.33–2.81)
Obesity	9 (8.3)	406.1	22.2	14 (2.4)	3157.6	4.4	4.85 (2.08–11.35)	5.56 (1.98–15.63)
Depression	45 (1.5)	11 480.6	3.9	76 (2.2)	19 003.3	4.0	0.97 (0.67–1.41)	1.48 (0.99–2.22)
Monthly income (NTD)								
≤19 999	270 (1.8)	76 606.6	3.5	494 (1.3)	219 989.1	2.2	1.57 (1.35–1.82)	2.04 (1.75–2.37)
20 000–39 999	33 (0.7)	22 103.4	1.5	83 (0.5)	87 295.4	1.0	1.55 (1.04–2.33)	1.95 (1.27–3.00)
≥40 000	8 (0.7)	6441.6	1.2	27 (0.4)	32 795.1	0.8	1.49 (0.68–3.28)	1.34 (0.58–3.12)
Follow-up period								
<1 mo	33 (0.2)	1693.8	19.5	8 (0.01)	5211.6	1.5	12.64 (5.84–27.35)	12.70 (5.73–28.12)
1–12 mo	61 (0.3)	17 438.1	3.5	97 (0.2)	54 805.3	1.8	1.98 (1.44–2.72)	2.41 (1.73–3.36)
1–2 y	27 (0.2)	16 931.7	1.6	85 (0.2)	54 069.9	1.6	1.01 (0.66–1.56)	1.17 (0.75–1.82)
≥2 y	190 (1.2)	69 088.1	2.8	414 (0.8)	225 992.9	1.8	1.50 (1.27–1.78)	1.85 (1.55–2.21)

AHR indicates adjusted hazard ratio; COP, carbon monoxide poisoning; HR, hazard ratio; and NTD, New Taiwan Dollars.

*Adjusted for sex, hypertension, diabetes, hyperlipidemia, ischemic heart disease, thyroid disease, alcoholism, chronic obstructive pulmonary disease, malignancy, drug abuse, obesity, depression, monthly income, and index year.

†Incidence per 1000 person-years.

a higher risk during the first month (<1 month) than that during subsequent follow-up (AHR, 2.17 [95% CI, 1.44–3.28]; Table I in the Data Supplement). The Kaplan-Meier method and the log-rank test showed a higher risk for CHF in the COP cohort (Figure 1). In the COP cohort, patients with acute respiratory failure (ie, more severe COP) had a higher risk (Figure I in the Data Supplement). We also conducted further analyses using propensity score matching and competing risk survival analysis to account for imbalances between the 2 cohorts and found similar results (Tables II and III in the Data Supplement).

Cox proportional hazards regression showed that COP, older age, hypertension, diabetes, ischemic heart disease, thyroid disease, chronic obstructive pulmonary disease, obesity, and lower monthly income were independent predictors for CHF in all the patients (Table 3). The competing risk survival analysis also showed similar results (Table IV in the Data Supplement).

On echocardiography, when compared with pre-COP, rats exhibited significant LV structural changes, such as increases in LV internal dimension in diastole and LV internal dimension in systole after COP (Figure 2). Functional abnormalities in post-COP rats were

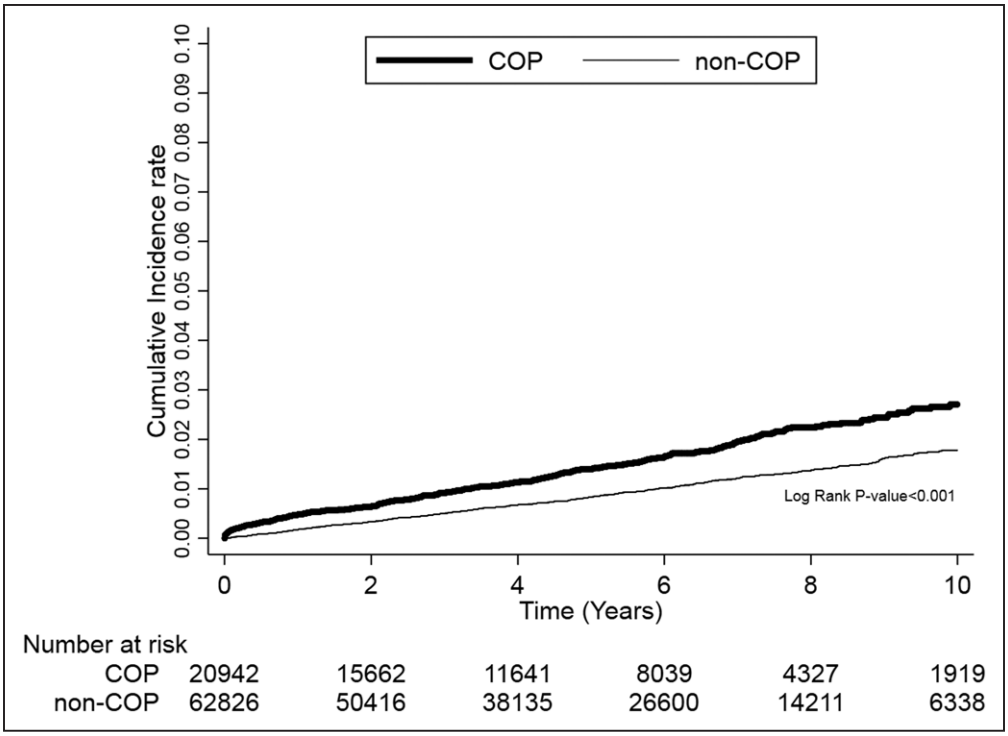

Figure 1. Comparison of the risk of congestive heart failure (CHF) between carbon monoxide poisoning (COP) and non-COP cohorts by the Kaplan-Meier method and the log-rank test.

in accordance with the structural changes. LVEF and LV fractional shortening were significantly decreased immediately after COP (day 1). The decreases persisted to day 28, except that changes in LVEF and LV fractional shortening on day 7 did not reach statistical significance. LV end-systolic volume increased immediately after COP (day 1), and the increase persisted to day 28, except that changes on day 7 did not reach statistical significance. Other echocardiographic parameters were shown in Figure II in the Data Supplement.

Compared with controls, rats in the COP group had similar values of all echocardiographic parameters at the beginning of study (Figure 2 and Figure II in the Data Supplement). Post-COP, structural changes observed using values of the controls as references were comparable with those when pre-COP values were used as references, namely larger LV internal dimension in diastole and LV internal dimension in systole. Functional abnormalities were also comparable with those observed using pre-COP values as references, namely significantly lower LVEF and LV fractional shortening except for on day 7, significantly larger LV end-systolic volume except for on day 7.

Histological examination with hematoxylin and eosin staining showed that in the cardiac tissue of post-COP rats, there were many necrotic cells with some neutrophils around them, myofilaments lined up loosely, and their nuclei translocated and even disappeared, which were different from the appearance in the sham group (Figure 3). On day 28 post-COP, collagen was deposited on the LV myocardial tissue (Figure 3D). Compared with the sham group, the myocardium damage scores and fibrosis area increased (Figure 3E and 3F). In addition, the cardiac toxic biomarker troponin-I increased on day 1 post-COP exposure and returned to the pre-COP level on the days 7, 14, 21, and 28 (Figure 3G).

DISCUSSION

The epidemiological study showed that patients with COP had a higher risk for CHF. The increased risk for CHF was highest in the first month of follow-up and persisted even after 2 years. Patients with COP with acute respiratory failure had a higher risk for CHF, suggesting a dose-response relationship between COP and CHF. The animal study confirmed that LVEF decreased after COP, supporting an increased risk for CHF. Cardiac pathology in rats on post-COP day 28 also revealed increased damage score and fibrosis area, comparable with findings in the epidemiological study and echocardiography examinations.

Several mechanisms might lead to cardiac dysfunction following COP. Hypoxia, due to decreased systemic oxygen delivery, plays the main role.[1] Animal studies revealed that initial COP-induced hypoxia was compensated for by increased cardiac output and oxygen extraction.[1,23] However, cardiovascular collapse and subsequent death developed when these compensatory mechanisms

Table 3. Independent Predictors of Congestive Heart Failure in All Patients by Cox Proportional Hazards Regression

Variable	Crude HR (95% CI)	Adjusted HR* (95% CI)
Cohort		
COP patients	1.66 (1.45–1.91)	2.01 (1.74–2.32)
Non-COP patients	1 (reference)	1 (reference)
Age, per 10 y	2.29 (2.21–2.38)	1.87 (1.78–1.95)
Sex		
Female	1 (reference)	1 (reference)
Male	1.15 (1.01–1.31)	1.08 (0.95–1.24)
Underlying comorbidity		
Hypertension	9.74 (8.51–11.15)	1.37 (1.15–1.63)
Diabetes	8.82 (7.51–10.36)	1.84 (1.53–2.21)
Hyperlipidemia	4.93 (4.16–5.85)	0.85 (0.69–1.03)
Ischemic heart disease	13.22 (11.56–15.12)	3.25 (2.77–3.82)
Thyroid disease	1.68 (1.31–2.15)	1.46 (1.13–1.87)
Alcoholism	1.86 (1.15–3.00)	0.96 (0.53–1.72)
Chronic obstructive pulmonary disease	13.47 (10.07–18.02)	1.36 (1.00–1.84)
Malignancy	4.13 (3.05–5.59)	1.13 (0.83–1.54)
Drug abuse	1.79 (1.12–2.85)	1.37 (0.77–2.44)
Obesity	3.22 (2.13–4.87)	2.39 (1.57–3.65)
Depression	2.08 (1.72–2.52)	1.19 (0.97–1.44)
Monthly income (NTD)		
≤19 999	2.87 (2.05–4.03)	1.86 (1.32–2.63)
20 000–39 999	1.19 (0.82–1.74)	1.48 (1.01–2.17)
≥40 000	1 (reference)	1 (reference)

COP indicates carbon monoxide poisoning; HR, hazard ratio; and NTD, New Taiwan Dollars.

*Adjusted for sex, hypertension, diabetes, ischemic heart disease, hyperlipidemia, thyroid disease, alcoholism, chronic obstructive pulmonary disease, malignancy, drug abuse, obesity, depression, monthly income, and index year.

were overwhelmed.[1,23] In addition to COP, environmental studies also showed that CO and other air pollutants might increase the risk for arterial and venous thrombosis.[1,24] A nationwide study in China found an increased mortality of 1.12% (95% posterior interval, 0.42–1.83) from cardiovascular disease associated with a 1 mg/m^3 increase in average CO concentrations on a day and the previous day.[25]

There are other mechanisms that may lead to cardiac dysfunction following COP. Coronary vasoconstriction and subsequent myocardial injuries increase due to endothelial dysfunction from CO and increased production of free radicals.[1,24] The risk of thrombosis increases due to CO binding to fibrinogen-bound heme and increased platelet aggregation.[1] During ischemia-reperfusion, CO may increase nitric oxide-induced myocardial injury by increasing inducible nitric oxide synthase expression.[26] The higher affinity for CO than oxygen in myocytes and CO-induced inhibition of oxidative phosphorylation causes myocardial infarction and subsequent cardiac dysfunction.[24,27] Similar to the effects of cyanide, CO inhibits mitochondrial respiration and shuts down oxidative phosphorylation, resulting in decreased ATP production in the heart.[1] COP activates platelets and then stimulates neutrophils to degranulate and release myeloperoxidase, which amplifies the inflammatory effects by triggering more neutrophil activation, adhesion, and degranulation.[1,28] Proteases from neutrophils oxidize endothelial cell xanthine dehydrogenase to xanthine oxidase and generate reactive oxygen species.[1,28] The inflammatory response introduced by nitric oxide and reactive oxygen species is a long-term effect for cardiac injury following COP.[1]

The possible explanations for the different results between the current study and previous studies[8,10] are the longer follow-up period, more participants, more comprehensive ascertainment of patients (inclusion of patients of COP who were later diagnosed with CHF at a different hospital), and more confounding factors adjusted. The study by Kalay et al[8] enrolled 20 patients and followed up them for 1 week only. The current study followed patients for at least 1 year and found the incidence rate of CHF was 1.5%. With this incidence rate, the study by Kalay et al would expect to observe only 0.3 cases of CHF even if the patients had been followed for >1 year. The study by Lee at al[10] identified 8381 patients with COP and adjusted for 4 comorbidities (diabetes, hypertension, hyperlipidemia, and chronic obstructive pulmonary disease) only. The current study identified 20 942 patients and adjusted for additional comorbidities (ischemic heart disease, thyroid disease, alcoholism, malignancy, drug abuse, obesity, and depression) and covariates (monthly income and index year), which may minimize confounding effects.

Although a higher risk for CHF was found in the COP cohort, the incidence of CHF was generally low (<10%) except in individuals ≥65 years. Therefore, a risk stratification incorporating the independent predictors for CHF for following up heart function in the patients with COP would be better than comprehensive screening on all the patients with COP.

The major strength of this study is the combination of epidemiological and animal studies. Additional strengths include that the epidemiological study is nationwide and has a large sample. The main limitation is that some confounding factors, including body mass index and smoking, are not available in the NHIRD. However, we think that confounding bias was minimized by adjustment for underlying comorbidities and other potential confounding factors as well as propensity matching. In particular, smoking is an important potential confounding factor, and we used 2 approaches to address its effects. One was that we included chronic obstructive pulmonary disease, which is closely related to smoking, as a surrogate indicator for smoking in the analysis and adjusted for its effects. The other was

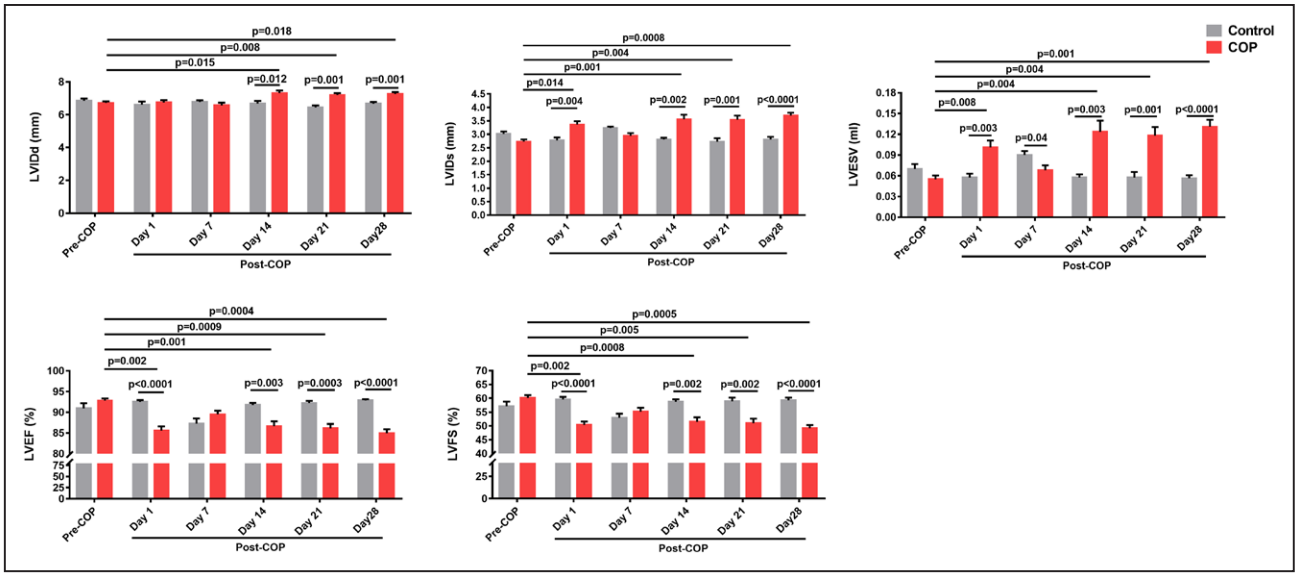

Figure 2. Comparisons of left ventricular (LV) volumes, contractility, and hemodynamic parameters calculated from echocardiographic measurements between carbon monoxide poisoning (COP) rats and pre-COP rats and between COP rats and control rats.

The LV internal dimension in diastole (LVIDd), LV internal dimension in systole (LVIDs), LV end-systolic volume (LVESV), left ventricular ejection fraction (LVEF), and LV fractional shortening (LVFS) were assessed via 2-dimensional echocardiography.

that we conducted stratified analyses by sex, and the prevalence of smoking in Taiwanese women is very low, <2.4% in 2018.[29] As shown by the results, the effects of COP on CHF were similar between Taiwanese men and women (AHR, 2.02 versus 2.07), and therefore, the effects of smoking were unlikely to affect our major conclusion. Second, we were not sure that all patients with COP had no CHF initially because echocardiography is not a routine examination of COP and the reports were not included in the NHIRD. However, we had excluded those who had acute pericarditis, acute and subacute endocarditis, acute myocarditis, other diseases of pericardium, other diseases of endocardium, cardiomyopathy, conduction disorders, cardiac dysrhythmias, heart failure, and ill-defined descriptions and complications of heart disease before the index date, which should minimize the probability of including such patients as study participants. In addition, even if some of the participants had CHF, we think the misclassification was nondifferential between the 2 cohorts, which is unlikely to introduce a misclassification bias. Third, the lack of echocardiography also limits the classification of heart failure with preserved ejection fraction versus heart failure with reduced ejection fraction. Further studies about the effects of COP on heart failure with preserved ejection fraction and heart failure with reduced ejection fraction separately are warranted to clarify this issue. Fourth, inaccurate (over or under) ascertainment of cases is a general limitation of studies using administrative-level data. However, the National Health Insurance of Taiwan has comprehensive coverage with a low premium and a high density of health care providers, and >99.9% of the citizens are enrolled. Therefore, cost and access to medical facilities, 2 major factors leading to under ascertainment in such studies, are unlikely to introduce remarkable effects on the results of our study. In addition, COP is a condition associated with a very specific toxic agent, and there is no conceivable incentive for making a false diagnosis of COP because the National Health Insurance covers all reasonable costs. Consequently, our study results are unlikely to be remarkably affected by over-diagnosis. Further prospective hospital-based studies may help confirm our findings. It should also be noted that in addition to hypoxia per se, COP may also lead to health effects through free radical generation, mitochondrial inhibition, and inflammation.[1,6] Therefore, further large-scale follow-up studies should be conducted to identify other long-term effects that have not been reported in the literature.

CONCLUSIONS

This combined epidemiological and animal study delineated that the risk for CHF increased following COP, was highest in the first month, and persisted even after >2 years. The risk of CHF increased as the severity of COP increased. The possible mechanisms include hypoxic injury, free radical generation, mitochondrial inhibition, and inflammation. We suggest following up patients with COP closely to assess cardiac function, especially in the patients with older age, hypertension, diabetes, and obesity. In addition to treating COP per se, managing the above-mentioned comorbidities may

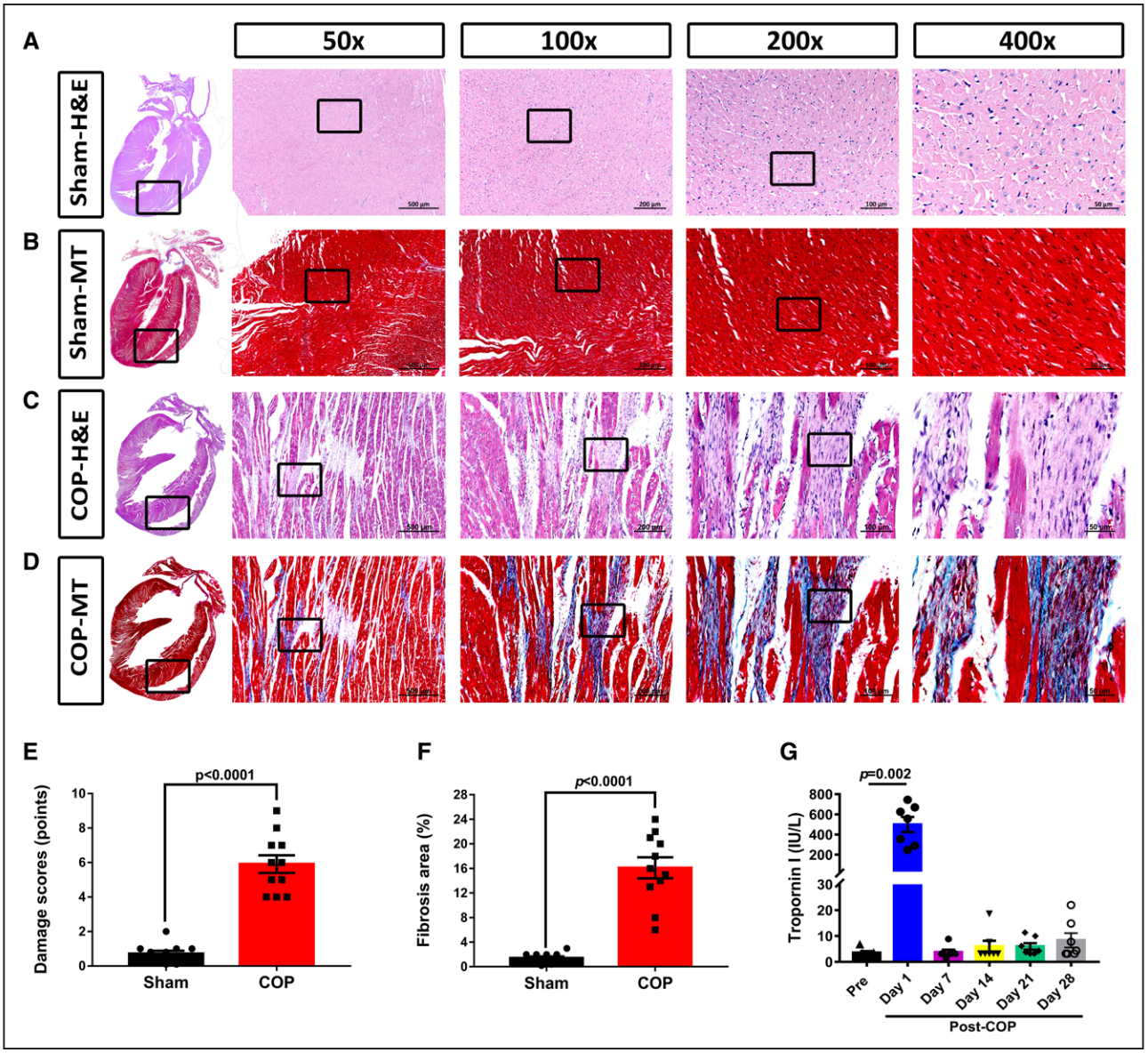

Figure 3. Heart section from a sham rat and a carbon monoxide poisoning (COP) rat under low (×50) to high (×400) magnification and cardiac toxic marker troponin-I measurement.
The morphological changes and cardiac fibrosis were analyzed using hematoxylin and eosin (H&E) and Masson trichrome (MT) staining. **A**–**D**, The picture shows low to high magnification of tissue structure, and blue areas show collagen deposition. **E**-**F**, Quantitative evaluation of heart damage score (**E**) and fibrotic score (**F**) in each group of rats. **G**, Troponin-I changes in the COP rats. Data are expressed as mean±SD. Statistical comparison was made using the independent Student *t* test.

also help to prevent the development of CHF. Our findings call for large-scale follow-up studies to identify other long-term effects of COP that have not been reported in the literature.

ARTICLE INFORMATION

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Drs Huang and Guo designed and conceived this study and wrote the article. T.-H. Chen and Dr Chang performed the animal study and statistical analysis and wrote the article. C.-H.H. and Y.-C. Chen performed the statistical analysis and wrote the article. Drs Hsu, Lin, and Wang provided professional suggestions and wrote the article. All authors read and approved the final article.

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Disclosures

None.

Supplemental Materials

Tables I–IV
Figures I–II

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