Endogenous Carbon Monoxide Production and Diffusing Capacity of the Lung for Carbon Monoxide in Sepsis-Induced Acute Respiratory Distress Syndrome

Low-dose inhaled carbon monoxide is a novel therapeutic under investigation in acute respiratory distress syndrome. The Coburn-Forster-Kane equation is a well-validated model of carbon monoxide uptake that can accurately predict carboxyhemoglobin levels to ensure safe administration of low-dose inhaled carbon monoxide in patients with acute respiratory distress syndrome. Using data from a Phase I trial of low-dose inhaled carbon monoxide, we performed a post hoc analysis to determine if the Coburn-Forster-Kane equation could be used to assess the diffusing capacity of the lung for carbon monoxide and endogenous carbon monoxide production in patients with sepsis-induced acute respiratory distress syndrome. Diffusing capacity of the lung for carbon monoxide was substantially reduced and correlated with PaO_2/FiO_2 and Sequential Organ Failure Assessment score. Endogenous carbon monoxide production was markedly elevated and was significantly associated with Lung Injury Score in sepsis-induced acute respiratory distress syndrome patients. Our data suggest that the Coburn-Forster-Kane equation can be used to estimate diffusing capacity of the lung for carbon monoxide and endogenous carbon monoxide production in mechanically ventilated patients. We found that increased endogenous carbon monoxide production and reduced diffusing capacity of the lung for carbon monoxide correlate with clinical endpoints associated with outcomes in patients with sepsis-induced acute respiratory distress syndrome.

To the Editor:

The therapeutic use of low-dose inhaled carbon monoxide (iCO) in sepsis-induced acute respiratory distress syndrome (ARDS) is currently being investigated in Phase I (NCT02425579) (1) and Phase II clinical trials (NCT03799874). We recently showed that the widely used Coburn-Forster-Kane (CFK) equation (2–4) accurately predicted carboxyhemoglobin (COHb) levels during iCO treatment ensuring safe administration in ARDS patients (1). However, it is unclear whether the CFK equation can be used to assess precisely the diffusing capacity of the lung for CO (D_lco) and the endogenous carbon monoxide production (VCO) in patients with ARDS. In young healthy adults, VCO is 0.007 ± 0.001 mL per minute standard temperature (0°C), standard pressure (760 mm Hg), and dry (5) and originates predominantly from heme breakdown by the enzyme heme oxygenase (HO). However, increased expression of the inducible isoform HO-1 (6) may lead to higher VCO in inflammatory lung diseases and critical illness (7, 8). This suggests that assessment of VCO may provide critical insights into inflammatory subphenotypes in ARDS that could contribute to precision-medicine approaches in the future. To estimate D_lco and VCO in mechanically ventilated ARDS patients and determine whether D_lco and VCO correlate with clinical endpoints, we used the CFK equation (2, 3) and time series data of COHb measurements obtained from ARDS subjects during iCO administration in our Phase I trial (1).

MATERIALS AND METHODS

We conducted a post hoc analysis of 25 iCO exposures in eight sepsis-induced ARDS subjects who were enrolled in our Phase I iCO trial (NCT02425579) (1). The original study was approved by the respective Institutional Review Boards prior to activation and individual informed consent was provided by all trial participants or their surrogates. Four subjects received iCO at 100 ppm and four subjects received iCO at 200 ppm for 90 minutes per day for up to 5 consecutive days. COHb levels were measured at baseline, 20, 60, 75, and 90 minutes for each subject on each day of iCO treatment. Exhaled CO was measured (NICO, Philips Respironics, Andover, MA) and used to calculate dead space fraction and alveolar ventilation (V_a) at baseline daily (1). The Sequential Organ Failure Assessment (SOFA) score and the Lung Injury Score (LIS), a composite 4-point scoring system including the extent of infiltrates on chest radiography, PaO_2/FiO_2, positive end-expiratory pressure, and respiratory system compliance, were calculated daily for each subject (1).

For each iCO exposure, the COHb levels at the five time points and measured subject parameters were used in the CFK equation (2, 3):

\[
(\dot{A}[HbCO] - B \dot{V}_{co} - P_{ICO})/(\dot{A}[HbCO] - B \dot{V}_{co} - P_{ICO}) = \exp(-t \lambda / \dot{V}_b) \cdot \exp(t / \dot{P}_b)
\]

where \(\dot{A} = P_{O_2} / M[HbO_2], B = 1/D_{lco} + P_tr/V_a, M = \) ratio of the affinity of blood for CO to that for O_2, \([HbO_2] = \) mL of O_2 per mL of blood, \([HbCO] = \) mL of CO per mL of blood at time \(t, [HbCO] = \) mL of CO per mL of blood at time \(0, P_{O_2} = \) average partial pressure of O_2 in lung capillaries; \(P_tr = \) barometric pressure minus the vapor pressure of water, \(\dot{V}_b = \) blood volume, \(P_{ICO} = \) partial pressure of CO in the inhaled air; and \(t = \) exposure duration.
Key parameters of the CFK equation include $\text{HbO}_2$, HbCO, and alveolar ventilation. Pulmonary capillary partial pressure of oxygen is assumed to be equal to its alveolar partial pressure rederived from the alveolar gas equation for oxygen linking it to Fio$_2$.

An iterative search was implemented as an optimization algorithm for the parameter estimation. The CFK model (3) and optimization were programmed using MATLAB (Mathworks, Natick, MA) as described (9). Total blood volume was estimated using the height cubed-body mass formula by Nadler et al (10) taking height, weight, and sex into account. Four models were investigated: 1) estimating both $D_{\text{Lco}}$ and VCO, 2) estimating $D_{\text{Lco}}$ using VCO = 0.007 mL/min and assuming no measurement error in COHb at baseline, 3) estimating $D_{\text{Lco}}$ and baseline COHb using VCO = 0.007 mL/min, and 4) estimating VCO using the subject’s predicted $D_{\text{Lco}}$ adjusted for hemoglobin. Additionally, we assumed a steady state for COHb prior to iCO exposure for CFK model A. Least-square curve fitting was performed to estimate the model parameters and determine the residuals as differences between the CFK model predictions and the measured COHb time series for the individual days of iCO exposure. The Bayesian information criterion (BIC) was calculated to identify the optimal model for COHb kinetics. For sensitivity analyses of $D_{\text{Lco}}$ and VCO estimates, the jackknife method was performed excluding one of the five times points per run. A linear mixed-effect model was used to evaluate the associations between daily VCO and LIS; VCO and SOFA score; $D_{\text{Lco}}$ and Pao$_2$/Fio$_2$; as well as $D_{\text{Lco}}$ and SOFA score using STATA software version 14.0 (StataCorp, College Station, TX).

Results

Model A of the CFK equation was selected as the best model based on the BIC (Supplemental Fig. 1, Supplemental Digital Content 1, http://links.lww.com/CCX/A427), mean root mean square errors (RMSEs) of COHb residuals (Supplemental Fig. 2, Supplemental Digital Content 1, http://links.lww.com/CCX/A427), and the convergence of $D_{\text{Lco}}$ (Supplemental Fig. 3, Supplemental Digital Content 1, http://links.lww.com/CCX/A427), and was thus used to estimate $D_{\text{Lco}}$ and VCO (9). The full dataset of the parameter identification for the 25 iCO exposures comparing the four CFK models and a jackknife analysis are available in Supplemental Table 1 (Supplemental Digital Content 2, http://links.lww.com/CCX/A428) (which includes data of CFK models A–D for the 25 iCO exposures) and Supplemental Table 2 (Supplemental Digital Content 2, http://links.lww.com/CCX/A428) (which includes data of the jackknife analysis for CFK model A).

The individual daily $D_{\text{Lco}}$ ranged from 0.37 to 8.24 mL/min/mm Hg, VCO ranged from 0.008 to 0.078 mL/min, and LIS ranged from 1.50 to 3.50. The clinical characteristics, CFK model parameters, and estimates of VCO and $D_{\text{Lco}}$ in ARDS subjects are summarized in Table 1. The VCO and $D_{\text{Lco}}$ differed among subjects and varied over time (Fig. 1, A and B). The RMSE of the residuals between model predictions and COHb measurements ranged from 0.006% to 0.347% COHb. Jackknife-based sensitivity analyses demonstrated that the uncertainty of most VCO and $D_{\text{Lco}}$ estimates was very small (Fig. 1, A and B). Furthermore, the CFK model

### TABLE 1. Clinical Characteristics and Coburn-Forster-Kane Model Parameters in Sepsis-Induced Acute Respiratory Distress Syndrome Subjects

| Age, Gender | Weight (kg) | Fio$_2$ | Pao$_2$/Fio$_2$ (mm Hg) | Dead Space Over Tidal Volume | Lung Injury Score | Sequential Organ Failure Assessment Score | Root Mean Square Error of Carboxy-hemoglobin Residual | Endogenous Carbon Monoxide Production (mL/min) | $D_{\text{Lco}}$ (mL/min/mm Hg) | Normal $D_{\text{Lco}}$ (mL/min/mm Hg)$^a$ |
|-------------|------------|---------|-------------------------|-----------------------------|------------------|-------------------------------------------|-----------------------------------------------|---------------------------------------------|---------------------------------|----------------------------------------|
| 100 ppm     |            |         |                         |                             |                  |                                           |                                               |                                              |                                 |                                       |
| Subject 1   | 68 M       | 68.8    | 0.40$^{0.00}$           | 277                         | 0.58$^{0.05}$    | 2.19$^{2.00}$                             | 10.5$^{12}$                                    | 0.152$^{0.055}$                           | 0.029$^{0.008}$                | 2.59$^{0.37}$                           | 22.96 $^b$                             |
| Subject 2   | 70 F       | 59.4    | 0.45$^{0.40}$           | 185                         | 0.65$^{0.07}$    | 2.38$^{2.00}$                             | 10.0$^{11}$                                    | 0.077$^{0.006}$                           | 0.043$^{0.037}$                | 4.94$^{3.76}$                           | 16.36 $^b$                             |
| Subject 3   | 63 F       | 55.7    | 0.40$^{0.40}$           | 156                         | 0.46$^{0.41}$    | 2.00$^{2.00}$                             | 9.5$^{10}$                                    | 0.056$^{0.053}$                           | 0.043$^{0.039}$                | 2.44$^{2.38}$                           | 17.29 $^b$                             |
| Subject 4   | 63 F       | 110.4   | 0.40                   | 284                         | 0.46$^{0.42}$    | 2.13$^{2.00}$                             | 11.0$^{13}$                                   | 0.102$^{0.056}$                           | 0.057$^{0.051}$                | 5.43$^{4.04}$                           | 22.89 $^b$                             |
| 200 ppm     |            |         |                         |                             |                  |                                           |                                               |                                              |                                 |                                       |
| Subject 5   | 45 M       | 61.7    | 0.69$^{0.60}$           | 166                         | 0.66$^{0.60}$    | 2.31$^{2.15}$                             | 6.8$^{8}$                                     | 0.045$^{0.006}$                           | 0.068$^{0.063}$                | 2.61$^{2.15}$                           | 29.22 $^b$                             |
| Subject 6   | 61 F       | 56.7    | 0.40                   | 298                         | 0.68$^{0.60}$    | 2.13$^{2.15}$                             | 11.5$^{12}$                                   | 0.096$^{0.006}$                           | 0.044$^{0.038}$                | 2.41$^{2.36}$                           | 19.48 $^b$                             |
| Subject 7   | 51 M       | 113.7   | 0.80                   | 147                         | 0.59$^{0.57}$    | 3.38$^{3.00}$                             | 13.0$^{14}$                                   | 0.048$^{0.006}$                           | 0.073$^{0.071}$                | 3.45$^{2.87}$                           | 21.15 $^b$                             |
| Subject 8   | 38 F       | 85.0    | 0.40                   | 203                         | 0.53$^{0.52}$    | 2.33$^{2.50}$                             | 5.0$^{6}$                                     | 0.052$^{0.039}$                           | 0.035$^{0.032}$                | 2.64$^{2.50}$                           | 22.16 $^b$                             |

$^a$Normal $D_{\text{Lco}}$ is predicted using Global Lung Function Initiative reference values.

Data are presented as mean $^{\text{max}}$.
performed well in subjects across a range of \( \text{FiO}_2 \) requirements and high dead space fractions. A linear mixed-effect model showed that increased daily \( \dot{V}_{\text{CO}} \) was significantly associated with increased LIS (\( p = 0.05 \)) and that decreased daily \( D_{\text{LCO}} \) significantly correlated with both decreased \( \text{Pao}_2/\text{FiO}_2 \) and increased SOFA score (\( p = 0.002 \) and 0.005, respectively) (Fig. 1, C and D). There was no association between \( \dot{V}_{\text{CO}} \) and SOFA score.

**DISCUSSION**

In a total of 25 iCO exposures in eight ARDS patients who had a variety of \( \text{FiO}_2 \) ranges and high dead space fractions, we show for the first time CFK-based estimates of \( \dot{V}_{\text{CO}} \) and \( D_{\text{LCO}} \) in ARDS where: 1) \( \dot{V}_{\text{CO}} \) was elevated and significantly correlated with LIS, 2) \( D_{\text{LCO}} \) was substantially lower than the normal predicted \( D_{\text{LCO}} \) and was significantly associated with \( \text{Pao}_2/\text{FiO}_2 \) and SOFA score, and 3) COHb predictions using the CFK equation fit well with the measured COHb.

Increased \( \dot{V}_{\text{CO}} \) and decreased \( D_{\text{LCO}} \) have been reported in prior studies in critically ill patients (7, 11). Using a CFK equation model and time series COHb data, we demonstrated that \( D_{\text{LCO}} \) and \( \dot{V}_{\text{CO}} \) can be estimated simultaneously and showed that the uncertainty from measurement errors in the COHb time series was remarkably small for most of the 25 iCO exposures. Our \( D_{\text{LCO}} \) estimations are similar to the results of Macnaughton et al (11) measured by the rebreathing method, which ranged from 0.18 to 6.72 mL/min/mm Hg. Additionally, our \( D_{\text{LCO}} \) estimations significantly correlated with \( \text{Pao}_2/\text{FiO}_2 \) and SOFA score. \( \dot{V}_{\text{CO}} \) is known to be elevated in mechanically ventilated patients with severe sepsis compared with ICU controls (7), but correlates poorly with Acute Physiology and Chronic Health Evaluation II and SOFA scores (8). This study reports for the first time a significant association between daily \( \dot{V}_{\text{CO}} \) and LIS in sepsis-induced ARDS patients.
Our study has several limitations. First, gold standard measurements of $D_{Lco}$ and VCO were not feasible during iCO administration in mechanically ventilated ARDS patients requiring high inspired oxygen concentration. Second, the 25 iCO exposures in eight subjects in this Phase I trial is a relatively small sample size warranting a larger follow-up study.

CONCLUSIONS

In conclusion, this study showed the feasibility of estimating $D_{Lco}$ and VCO using the CFK equation in ARDS patients and found that both $D_{Lco}$ and VCO significantly correlated with clinical endpoints in critical illness. Future studies are necessary in order to determine the clinical utility of $D_{Lco}$ estimation as a physiologic assessment of gas-exchange abnormalities in patients with hypoxic respiratory failure. An ongoing Phase II clinical trial of low-dose iCO in ARDS (NCT03799874) will further validate the prognostic value of VCO as a biomarker in ARDS.

Drs. Fredenburgh and Winkler contributed equally.

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