Increased heme catabolism in critically ill patients: correlation among exhaled carbon monoxide, arterial carboxyhemoglobin, and serum bilirubin \( \text{IX}\alpha \) concentrations

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Morimatsu, Hiroshi, Toru Takahashi, Kyoichiro Maeshima, Kazuyoshi Inoue, Tomoko Kawakami, Hiroko Shimizu, Mamoru Takeuchi, Masataka Yokoyama, Hiroshi Katayama, and Kiyoshi Morita. Increased heme catabolism in critically ill patients: correlation among exhaled carbon monoxide, arterial carboxyhemoglobin, and serum bilirubin \( \text{IX}\alpha \) concentrations. Am J Physiol Lung Cell Mol Physiol 290: L114–L119, 2006. First published August 12, 2005; doi:10.1152/ajplung.00031.2005.—It has been reported that exhaled carbon monoxide (CO) concentrations and arterial carboxyhemoglobin (CO-Hb) concentration in blood may be increased in critically ill patients. However, there was no study that examined correlation among amount of CO in exhaled air, CO-Hb concentrations in erythrocytes, and bilirubin IX\( \alpha \) (BR) in serum, i.e., the three major indexes of heme catabolism, within the same subject. Here, we examined CO concentrations in exhaled air, CO-Hb concentrations in arterial blood, and BR levels in serum in 29 critically ill patients. Measurements of exhaled CO, arterial CO-Hb, and serum total BR have been done in the intensive care unit. As control, exhaled CO concentration was also measured in eight healthy volunteers. A median exhaled CO concentration was significantly higher in critically ill patients compared with control. There was significant correlation between CO and CO-Hb and CO and total BR level. We also found CO concentrations correlated with indirect BR but not direct BR. Multivariate linear regression analysis for amount of exhaled CO concentrations also showed significant correlation with CO-Hb and total BR, despite the fact that respiratory variables of study subjects were markedly heterogeneous. We found no correlation among exhaled CO, patients’ severity, and degree of inflammation, but we found a strong trend of a higher exhaled CO concentration in survivors than in nonsurvivors. These findings suggest there is an increased heme breakdown in critically ill patients and that exhaled CO concentration, arterial CO-Hb, and serum total BR concentrations may be useful markers in critically ill conditions.

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IT HAS BEEN REPORTED that exhaled carbon monoxide (CO) concentrations (14, 26, 27) or carboxyhemoglobin (CO-Hb) concentration in blood (6, 10, 11) may be increased in critically ill patients. As CO is one of the metabolites of heme catabolism, it was suggested that there may be increased heme catabolism in these patients. Heme breakdown is catalyzed by microsomal heme oxygenase (HO), and, among its two isozymes, HO-1 is known to be inducible by various oxidative stresses, including surgery and inflammation (17, 19, 20). In contrast, HO-2 is expressed in a constitutive fashion and not influenced by these stimuli (8). HO-1 induction leads to increased heme breakdown, resulting in the production of iron, CO, and biliverdin \( \text{IX}\alpha \), which is then reduced to bilirubin \( \text{IX}\alpha \) (BR) by biliverdin reductase (15). Although CO or CO-Hb concentrations were studied in critically ill patients, there has been no study that examines correlation among the amount of CO in exhaled air, CO-Hb concentrations in erythrocytes, and serum BR concentrations simultaneously within the same subject. For these reasons, there has been substantial controversy concerning whether exhaled CO can be used as a biomarker in the diagnosis of medical intervention as well as in the prediction of outcomes (12). It is also known that previous studies suffer from technical artifacts in the measurement of arterial CO-Hb concentrations using spectrophotometry (22). In this study, we determined exhaled CO concentrations, CO-Hb in arterial blood by an improved technique with high specificity, and total BR levels in serum in 29 critically ill patients and examined their relationship. We report here that exhaled CO concentrations are significantly increased in critically ill patients compared with those in healthy volunteers. Exhaled CO concentration is also significantly correlated to arterial CO-Hb concentrations and serum total BR concentrations, despite the fact that the respiratory conditions of these patients were markedly variable. These findings thus suggest that there is an increased heme breakdown in critically ill patients and that increased exhaled CO, arterial CO-Hb, and serum total BR concentrations may be novel and useful markers of critically ill conditions.

MATERIALS AND METHODS

Patients and healthy volunteers. This study was conducted in conformity with the Declaration of Helsinki and was approved by the Institutional Review Board at Okayama University Medical School. After receiving the institutional approval and informed consent from study subjects, we consecutively studied 29 critically ill patients (Table 1). All patients were mechanically ventilated with either synchronized intermittent mandatory ventilation or pressure support ventilation by a Puritan-Bennett 7200 ventilator (Puritan-Bennett, Carlsbad, CA) through an endotracheal tube. Patients aged <18 yr and current smokers were excluded from the study. Respiratory rate (RR) and inspired oxygen fraction (\( \text{FI}_{\text{O}}_{2} \)) were obtained from the ventilator. Then, \( \text{Pa}_{2}/\text{FI}_{\text{O}}_{2} \) ratio (P/F ratio) was calculated. Daily sequential organ failure assessment (SOFA) score (21) was also calculated to assess patients’ severity. All measurements were done each morning in the intensive care unit (ICU) until extubation of the...
tracheal tube. As control, eight healthy volunteers were also included in this study. Written informed consent was also obtained from these volunteers. Nonsmokers without respiratory infection within 2 wk were used for healthy volunteers.

**Exhaled CO and carbon dioxide measurement.** Exhaled CO concentrations were measured using the newly developed CO analyzer (CARBOLYZER mBA-2000; TAIYO Instruments, Osaka, Japan). This machine includes a gas sensor adapted from a controlled potential electrolysis method (13). There are three electrodes, a working electrode, a counter electrode, and a reference electrode, in contact with the electrolytic liquid phase and gas phase by gas-permeable membrane (Fig. 1). When CO was exposed to the working electrode, an anode reaction took place as shown in chemical Eq. 1. At the counter electrode, in contact with oxygen in air, a cathode reaction as shown in chemical Eq. 2 took place. Thus, overall, reaction occurs as shown in chemical Eq. 3. Then, the CO concentration can be measured by sensing the oxidation reaction current.

\[
\begin{align*}
    \text{CO} + \text{H}_2\text{O} &\rightarrow \text{CO}_2 + 2\text{H}^+ + 2e^- \\
    \frac{1}{2}\text{O}_2 + 2\text{H}^+ + 2e^- &\rightarrow \text{H}_2\text{O} \\
    \text{CO} + \frac{1}{2}\text{O}_2 &\rightarrow \text{CO}_2
\end{align*}
\]

The CO analyzer, with sensitivity to 0.1 ppm, was calibrated weekly with a mixture of 45.4 ppm CO in air. The analyzer has the capability of continuous side-stream sampling with a rate of 0.2 l/min. A sampling adaptor was attached to the respiratory circuit for exhaled air sampling. We measured the exhaled CO concentrations continuously for 1 min in every measurement and calculated mean exhaled CO concentrations using a computerized analysis software (CARBOLYZER DataBox, TAIYO Instruments, Ina, Japan). Because lung CO excretion has been reported to be transiently dependent on FiO₂, we measured exhaled CO concentration during the period that the ventilation setting had remained unchanged for at least 7 h (26). Simultaneously, exhaled carbon dioxide (CO₂) concentrations were also measured by nondispersive infrared absorption sensors equipped with the same machine.

For healthy volunteers, the same ventilator as patients used was available, and a smoking control method was used to assess the relationship among exhaled CO concentration, arterial CO-Hb, and serum BR levels. The BR concentrations were also determined for 1 min in healthy volunteers similar to patients.

**Arterial CO-Hb measurement.** Arterial CO-Hb concentration was measured with a cooximeter blood gas analyzer (ABL 735; Radiometer Medical, Copenhagen, Denmark). This machine was specially calibrated and adjusted for CO-Hb wavelength (SAT 100), thereby achieving the most reliable results (23). The SAT 100 calibration requires heparinized blood samples that have to meet strict criteria on pH, PO₂, PO₂, hemoglobin, fraction of CO-Hb, and fraction of methemoglobin before this procedure is performed (22). The adjustment was carried out and verified by the manufacturer’s service organization. Routine arterial blood gas was measured at the same time of the measurements of exhaled CO concentrations. Other blood gas variables were also obtained from the same measurements.

**Serum BR concentrations.** Serum was separated from whole blood by centrifugation at 1,600 g for 10 min at 4°C, and serum BR concentration was measured using an automatic biochemical analyzer calibrated with quality control standards (Clinical Analyzer 7350; Hitachi High-Technologies, Tokyo, Japan). Simultaneously, concentrations of direct BR, equated with conjugated BR, were measured using the same machine, and then concentrations of indirect BR, equated with unconjugated BR, were calculated as follows [(indirect BR) = (BR) − (direct BR)]. White blood cell (WBC) counts and C-reactive protein (CRP) levels were also obtained from routine laboratory results to assess the degree of inflammation.

**Statistical analysis.** Data are presented as medians with interquartile range. Mann-Whitney’s test was used for a comparison between critically ill patients and healthy volunteers. Correlations in each variable were assessed with Pearson’s correlation coefficient and expressed in r² and P values. Multivariate linear regression analysis was used to assess the relationship among exhaled CO concentration, arterial CO-Hb, and serum BR levels taking respiratory variables into account. P < 0.05 was considered statistically significant.

**RESULTS**

We studied 29 consecutive critically ill patients. Table 1 shows the characteristics of these patients and reasons for their ICU admission. Surgical patients were predominant in our patient population. All patients were mechanically ventilated, and 66% of them were on inotropes and/or vasopressors. Three patients died in the hospital, thus hospital mortality was 10.3%.

Sixty-one paired samples for the measurements were obtained from 29 patients. A median exhaled CO concentration was significantly higher in critically ill patients compared with the control [median 2.90; interquartile range (1.60, 3.95) ppm vs. 1.75 (1.23, 2.00) ppm, respectively; P = 0.04; Fig. 2]. When we analyzed only the first measurements of exhaled CO (n = 29), it still revealed a significant higher level in critically ill patients.

![Figure 1. Structure of carbon monoxide (CO) sensor is shown. OP Amp, operational amplifier.](http://ajplung.physiology.org/)
ill patients compared with the control [3.40 (2.60, 4.35) ppm; P = 0.0021 compared with the healthy control]. Table 2 shows the measured variables in critically ill patients. Even though all our patients were mechanically ventilated, their respiratory status was relatively stable [RR 14 (12, 22) breaths/min; P/F ratio 288 (202, 399); PaCO2 41.0 (37.3, 45.6) mmHg].

There was significant correlation between CO and CO-Hb (r² = 0.20, P = 0.0008) and CO and total BR level (r² = 0.17, P = 0.001). Furthermore, CO correlated to indirect BR (r² = 0.23, P = 0.0006), but not to direct BR (r² = 0.002, P = 0.75). Even if we excluded the extreme values of BR (total BR > 5 mg/dl, indirect BR > 2 mg/dl), exhaled CO concentration was still significantly correlated with total BR (r² = 0.10, P = 0.016) and indirect BR (r² = 0.16, P = 0.0066). We further analyzed these correlations, dividing patients into two groups, i.e., surgical admission and medical admission. These analyses revealed that correlation between exhaled CO and indirect BR was steeper in the medical group than the surgical group, although other correlations were almost identical between the groups (Figs. 3 and 4). In addition, three respiratory variables were also significantly correlated with exhaled CO concentrations (RR: r² = 0.45, P < 0.0001; exhaled CO2: r² = 0.38, P < 0.0001; and P/F ratio: r² = 0.095, P = 0.019), which suggested that patients’ respiratory status could influence the concentrations of exhaled CO. However, multivariate linear regression analysis for the amount of exhaled CO concentrations showed significant correlation with CO-Hb and total BR, regardless of their various respiratory indexes (Table 3).

Exhaled CO concentrations did not correlate with patients’ severity (SOFA score) and the degree of inflammation (SOFA score: r² = 0.002, P = 0.71; WBC: r² = 0.004, P = 0.64; CRP: r² = 0.016, P = 0.34). However, we found that surgical patients had a significantly higher exhaled CO concentration than medical patients [3.50 (2.30, 4.30) ppm vs. 1.25 (0.80, 1.98) ppm; P < 0.0001]. We also found survivors had a higher exhaled CO concentration than nonsurvivors [3.9 (2.9, 4.5) ppm vs. 2.4 (0.8, 3.4) ppm; P = 0.11], although it did not reach statistical significance.

**DISCUSSION**

We found that the concentration of exhaled CO was significantly increased in critically ill patients compared with that in healthy volunteers. The increased CO concentration was well correlated with arterial CO-Hb, serum total BR, and indirect BR, despite the fact that respiratory status was markedly variable. These findings suggest that heme catabolism may be increased in critically ill patients, probably due to systemic oxidative stress.

As shown in Fig. 2, CO was only barely detectable in the exhaled air of healthy volunteers. In contrast, exhaled CO concentrations in critically ill patients were significantly increased compared with those of healthy volunteers. All of the critically ill patients in our study were mechanically ventilated through an endotracheal tube that bypassed the upper airways. Thus, we believe that exhaled CO concentrations were principally derived from lung alveoli, but not from the nonconducting airways. Exhaled CO concentration has been reported to be increased in critically ill patients (14, 26, 27). We have also reported that exhaled CO concentration was increased after.

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**Table 2. Measured variables in critically ill patients**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Median (IQR)</th>
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<tr>
<td>Exhaled CO, ppm</td>
<td>2.90 (1.60, 3.95)</td>
</tr>
<tr>
<td>%Exhaled CO2</td>
<td>2.00 (2.65, 3.60)</td>
</tr>
<tr>
<td>%Arterial CO-Hb</td>
<td>1.40 (1.20, 1.65)</td>
</tr>
<tr>
<td>Respiratory rate, /min</td>
<td>14 (12, 22)</td>
</tr>
<tr>
<td>Serum total bilirubin, mg/dl</td>
<td>0.89 (0.57, 1.86)</td>
</tr>
<tr>
<td>Serum direct bilirubin, mg/dl</td>
<td>0.45 (0.23, 0.94)</td>
</tr>
<tr>
<td>Serum indirect bilirubin, mg/dl</td>
<td>0.56 (0.36, 0.92)</td>
</tr>
<tr>
<td>P/F ratio</td>
<td>288 (202, 399)</td>
</tr>
</tbody>
</table>

CO-Hb, carboxyhemoglobin; P/F ratio, PaO2/FIO2 ratio; IQR, interquartile range.
surgery, regardless of anesthetic techniques employed (4). Eighty-three percent of our patients were surgical, and 66% were on inotropes and/or vasopressors. Our patients also had signs of organ failure (median SOFA score 5). Thus, although the hospital mortality was 10.3%, all patients in our study were critically ill, and they were associated with increased exhaled CO concentration, increased arterial CO-Hb concentration, and increased serum BR concentration.

Our study demonstrated a significant correlation among the three major indexes of heme catabolism, i.e., CO concentrations in exhaled air, CO-Hb concentration in arterial blood, and total BR concentration in serum. To our knowledge, this study demonstrated for the first time, that there is correlation among the three indexes of heme catabolism. A previous study reported, however, that although exhaled CO concentration was increased in critically ill patients, it did not correlate with arterial and venous CO-Hb concentration (14). Thus this finding is in direct conflict with our findings. The authors of the previous study (14) used, however, an older version of the blood gas analyzer, which is much less sensitive than our analyzer, and was shown to significantly underestimate the arterial CO-Hb concentration (23). In contrast, we used one of the most recent versions of the blood gas analyzer with excellent sensitivity (25 times more sensitive than the older method) and with calibration of the wavelength specific for CO-Hb, which allowed accurate determination of CO-Hb (16). Thus these differences appear to have played a role in the different observations between the previous study (14) and our present study and point to the fact that highly specific detection is necessary for the determination of arterial CO-Hb concentration.

Another important finding is that exhaled CO concentrations are correlated with serum indirect BR, but not with serum direct BR. Indirect BR, produced by heme breakdown in the peripheral tissues, is bound tightly to albumin in the circulation and transported to the liver. Indirect BR most likely enters the hepatocyte and then is conjugated to glucuronic acid catalyzed by glucoronyl transferase to yield direct BR, which was excreted into the bile canaliculi. A small amount of the direct BR escapes into the blood and is excreted into the urine. It has been reported that exhaled CO can reflect bilirubin production in premature babies with neonatal hyperbilirubinemia (1, 7). These results suggest that exhaled CO concentrations reflect increased heme breakdown due to hemolysis. Thus our findings in critically ill patients also suggest that there may be increased extracorpuscular heme catabolism, leading to the induction of HO-1 and resultant production of CO and indirect

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**Table 3. Multivariate linear regression analysis for exhaled CO concentration**

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>%Arterial CO-Hb</td>
<td>1.22</td>
<td>0.43 to 2.00</td>
<td>0.0038</td>
</tr>
<tr>
<td>Serum total bilirubin, mg/dl</td>
<td>0.22</td>
<td>0.02 to 0.43</td>
<td>0.038</td>
</tr>
<tr>
<td>%Exhaled CO</td>
<td>0.52</td>
<td>0.15 to 0.88</td>
<td>0.0076</td>
</tr>
<tr>
<td>Respiratory rate /min</td>
<td>-0.10</td>
<td>-0.15 to -0.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>P/F ratio</td>
<td>0.0007</td>
<td>-0.002 to 0.003</td>
<td>0.59</td>
</tr>
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95% CI, 95% confidence interval.
BR. In addition to indirect BR, our findings showed that exhaled CO concentrations also correlated with total serum BR, suggesting that increased total serum BR concentrations in critically ill patients are largely due to indirect BR rather than to direct BR. Moreover, even if we exclude extreme BR values (total BR > 5 mg/dl, indirect BR > 2 mg/dl), exhaled CO concentrations still have good correlations with serum total BR and indirect BR. These additional results thus strongly substantiate the fact that exhaled CO levels reflect increased heme breakdown even in the physiological range of BR concentrations.

Previous studies also reported the increased exhaled CO concentrations in airway inflammatory disease (5, 24, 25). On the basis of these findings, it was suggested that inflammatory reaction in the lung may have been responsible for HO-1 induction in the lung, and the increase in exhaled CO concentration may be directly derived from the lung. Although it may be so in airway inflammatory diseases, increased exhaled CO concentrations in our study were observed in all critically ill patients independently of their abnormal respiratory indexes. Only 11 samples of our measurements were associated with P/F ratio <200, and only 2 patients were admitted to the ICU for respiratory reasons. Thus our findings strongly suggest that exhaled CO concentration can be increased in systemic stress responses, independently of local pulmonary inflammations. This point is also consistent with our previous observations that exhaled CO concentrations, as well as arterial CO-Hb concentrations, were significantly increased after surgery under both spinal anesthesia and general anesthesia with mechanical ventilation through an endotracheal tube (4). All these findings suggest that systemic stress responses, such as critically ill conditions, may elicit HO-1 induction in various organs, which then results in increases in exhaled CO concentration, arterial CO-Hb concentration, and serum total BR concentration (4). These findings also indicate that exhaled CO concentration, arterial CO-Hb concentration, and serum total BR concentration can be used as a useful measure of critically ill conditions.

HO-1 is known to be induced not only by its substrate, heme, but also by a variety of factors, such as heavy metals, inflammatory cytokines, and oxidative stress. Previous studies showed that HO-1 can be induced by oxidative stress and results in the production of iron, BR, and CO. It is known that endogenously produced CO is principally derived from heme catabolism by HO reaction (9, 19). We also demonstrated that HO-1 was induced in the lung as well as in the kidney, the liver, the intestine, and the brain in a rat model of sepsis (2, 3, 18), suggesting that HO-1 may be inducible in various organs in massive inflammation such as sepsis. Thus we speculate that systemic inflammation caused by critical illness may be responsible for HO-1 induction and results in the production of endogenous CO, ultimately resulting in an increase in CO concentration in exhaled air from the lung.

To explore the clinical significance of the measurement of exhaled CO concentration, we analyzed the relationship between exhaled CO concentrations and severity score (SOFA score) and the indexes of inflammation (WBC and CRP). There was no correlation between CO and these variables, suggesting that exhaled CO concentration is not directly correlated with either disease severity or the degree of inflammation. On the other hand, when the relationship of exhaled CO concentrations with other indexes was analyzed in two groups of patients, i.e., medical and surgical admission, there were similar correlation coefficients between the two groups for CO-Hb, total BR, and direct BR. In contrast, correlation curves between exhaled CO concentrations and indirect BR levels were quite different between the two groups. Namely, the medical patients had a steeper correlation between exhaled CO and indirect BR than the surgical patients, suggesting that, in addition to surgery itself, there were probably other stresses that led to increased heme breakdown in the surgical patients (Figs. 3 and 4). We also compared exhaled CO concentrations between survivors and nonsurvivors. Interestingly, it was found that survivors tended to have higher exhaled CO concentrations than nonsurvivors, but the difference did not reach statistical significance because of a limited sample size. These results suggest, however, that the poorer outcome of nonsurvivors may be due to their limited capacity in producing CO or in inducing HO-1. We believe that this aspect should be pursued by a future study.

In conclusion, our findings in this study demonstrated that there is a significant increase in exhaled CO concentration in critically ill patients compared with those of healthy volunteers. Increased CO concentration in exhaled air is also significantly correlated to increases in arterial CO-Hb and serum total BR concentrations, suggesting observed increases in CO, CO-Hb, and BR are due to increased heme catabolism in these patients. These findings thus suggest that there is an increased heme breakdown in critically ill patients and that increased exhaled CO, arterial CO-Hb, and serum total BR concentrations may be novel and useful markers of clinical severity of critical illnesses. Exhaled CO concentration may be of particular interest since it can be determined by a noninvasive measurement in a consecutive fashion.

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