CASE

A 46-year-old woman was found dead in the basement of her home after a fire. External examination of the body showed a wound to the head and soot in the nose and mouth, with soot also found internally in the trachea and bronchi upon autopsy. On questioning by the police, the woman’s husband admitted that he and his wife were having an argument that led to him accidentally knocking her unconscious by pushing her and causing her head to strike an object. The husband, believing her dead, then set fire to the house to hide his wife’s death. Police charged the husband with first-degree intentional felony murder (intentionally causing death while committing or attempting to commit arson) and second-degree unintentional felony murder (unintentionally causing death while committing a felony).

The prosecuting lawyers’ case hinged on the measured carboxyhemoglobin (COHb) concentration to prove that the wife was indeed alive when the fire was started and therefore the husband was also guilty of murder by committing arson. The blood COHb concentration measured by a 6-wavelength CO-oximeter was 61.4%. Defense lawyers argued that CO-oximetry was an unreliable method for postmortem COHb measurement, compared with other methods, and therefore the result obtained was not valid proof “beyond a reasonable doubt” that arson was the cause of the wife’s death.

DISCUSSION

CO POISONING

Exogenous CO is a by-product of the incomplete combustion of hydrocarbons. Poisoning by CO often goes undetected because of its lack of taste and odor. Endog-

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4 Nonstandard abbreviations: COHb, carboxyhemoglobin; GC, gas chromatography; MetHb, methemoglobin.

QUESTIONS TO CONSIDER

1. Was the victim’s COHb concentration at autopsy lethal?
2. Is CO-oximetry a reliable method for postmortem COHb measurement compared with gas chromatography (GC) and UV spectrophotometry?
3. What mechanisms are responsible for increases in methemoglobin (MetHb), and do increased concentrations interfere with CO-oximetry measurements of COHb?

enous CO is also produced naturally in the body. The pathogenesis of exogenous CO toxicity is its propensity to attach to the iron moiety of the heme group of hemoglobin. CO has a 210-fold higher affinity for hemoglobin compared with oxygen. CO binding also prevents hemoglobin from acquiring CO2 from tissues for removal and stabilizes oxygen molecules bound to the same hemoglobin protein, preventing their release to tissues. Organs with the highest oxygen demand, such as the heart and brain, are most affected by CO poisoning. The heart is also susceptible to the binding of CO to myoglobin, where it impairs the supply of oxygen to mitochondria. In a study of 230 patients with moderate to severe CO poisoning, 37% had ischemic electrocardiographic changes and increased concentrations of cardiac biomarkers (1). The symptoms of CO toxicity include headaches, dizziness, weakness, shortness of breath, and nausea. These general, nonspecific symptoms can cause doctors to miss CO poisoning. CO poisoning should be treated immediately with 100% oxygen to reduce the 300-min half-life of COHb to 90 min. More severe cases with COHb concentrations >25% and loss of consciousness should be treated with hyperbaric oxygen therapy to further reduce the half-life to 30 min.

WAS THE VICTIM’S COHb CONCENTRATION AT AUTOPSY LETHAL?

Measurement of COHb is crucial to recognizing CO as a contributor in deaths involving fires, exposure to automobile exhaust, aircraft accidents, and residential exposures. Knowledge of COHb concentrations assists
medical examiners in determining whether a victim was alive or dead when a fire started and multiple life-threatening injuries were present, as in the current case. The toxic effects of CO depend on the length of exposure, the concentration of CO gas, and ventilation. Short exposures to high CO concentrations, even greater than those typically associated with death, are often more survivable than exposures to more moderate concentrations over a prolonged period of time (2). Blood COHb concentrations ≤3% are found in nonsmokers, whereas smokers may have concentrations upwards of 10%–15%. In toxicologic investigations of cause of death, COHb concentrations >50% are considered lethal. A number of other factors, including declining health of the elderly, increased vulnerability of an infant, coronary artery disease, and respiratory insufficiency, can cause death at COHb concentrations <50% (2). In the current case, the high COHb concentration of 61%, with the findings of soot in the mouth, nose, and respiratory tract, led the medical examiner to conclude that the victim was alive before the fire was set and probably died from asphyxiation.

IS CO-OXIMETRY A RELIABLE METHOD FOR POSTMORTEM COHb MEASUREMENT, COMPARED WITH GC OR UV SPECTROPHOTOMETRY?
The validity of CO-oximetry for COHb measurement in postmortem samples has been examined by comparing CO-oximetry with UV spectrophotometry and GC. Interferences, including lipid-caused turbidity, MetHb, sulfhemoglobin, microcoagulates, putrefaction, and contamination, have called into question the accuracy of COHb measurements obtained by CO-oximetry. Older CO-oximetry technology with fewer monitored wavelengths often gave inaccurate COHb measurements in the presence of interferents, a limitation that has been improved with the availability of CO-oximeters with ≥6 wavelengths that correct for multiple types of interferents. Treatment with sodium dithionite to reduce MetHb and oxyhemoglobin, filtration to remove particulates, or other methods of pretreatment aid in making postmortem samples more suitable for CO-oximetry (3, 4). Use of CO-oximetry technology with >4 wavelengths improves correlations with GC results in postmortem samples, even at very low hemoglobin concentrations [<40 g/L (<4 g/dL)] (5). COHb measured on a CO-oximeter after treatment of putrefying blood samples to remove interference caused by high MetHb, sulfhemoglobin, turbidity, or low total hemoglobin correlated well with flagged CO-oximeter results before treatment and with GC results (6, 7).

In the current study, we compared COHb results obtained with automated CO-oximetry (Diametrics Medical AVOX 4000) and manual UV spectrophotometry (Hewlett Packard 8453 UV spectrophotometer) (8). Postmortem heart blood samples (EDTA anticoagulated) from 16 medical examiner cases were studied (Table 1). Before spectrophotometry, samples were treated with sodium dithionite. We measured the absorbance at 540 nm (COHb) and 555 nm (isosbestic point) and calculated the percent COHb concentration. The postmortem interval between death and blood draw ranged from 0 to 25.5 h. COHb concentrations ranged between 21% and 83%. Deming regression analysis of COHb data obtained by both CO-oximetry and UV spectrophotometry demonstrated an excellent correlation \( r = 0.983 (y = 1.04x - 1.21); S_y|x = 3.45; \) Fig. 1]. Neither the postmortem interval nor evidence of body decomposition (both of which are known to increase MetHb concentration) affected the correlation of the CO-oximeter and spectrophotometer results. These results demonstrate that the measurement of COHb by CO-oximetry (with an appropriate number of wavelengths) can be a valid and accurate method to assess CO in postmortem blood samples obtained in forensic toxicology cases.

WHAT MECHANISMS ARE RESPONSIBLE FOR INCREASES IN MetHb, AND DO INCREASED CONCENTRATIONS INTERFERE WITH CO-OXIMETRY MEASUREMENTS OF COHb?
Increased temperature causes oxidation of hemoglobin to MetHb (Fe\(^{2+}\) to Fe\(^{3+}\)), shifting the oxygen dissociation curve such that oxygen affinity is increased with less oxygen released to tissues. Victims caught in fires may have increased MetHb concentrations caused by the inhalation of nitrogen oxides produced by burning plastics (9). One must also be aware of possible postmortem increases in MetHb due to heat, especially concurrent with low to moderate levels of COHb or bacteria (10). In conjunction with burn and soot findings, measurements of MetHb, COHb, oxygenated hemoglobin, and deoxygenated hemoglobin are helpful in determining the cause of death in fire deaths. When CO-oximetry is used to measure COHb concentrations, we recommend that the manufacturer’s package insert be read thoroughly to ensure that sample handling and technology are adequate to address MetHb concerns.

CASE RESOLUTION

The jury found the husband guilty of second-degree unintentional felony murder. On appeal of the conviction, the defendant challenged the validity of the CO-oximetry results. During the pretrial to determine the admissibility of the CO results, a clinical chemist provided expert testimony that the CO-oximetry results obtained with >4-wavelength technology showed no MetHb interference. On the basis of this testimony, the court ruled that the results were valid and therefore were admissible during the trial.
<table>
<thead>
<tr>
<th>Case no.</th>
<th>COHb by CO-oximetry, %</th>
<th>COHb by spectrophotometry, %</th>
<th>Age, y</th>
<th>Sex</th>
<th>Race</th>
<th>Manner of death&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Cause of death</th>
<th>Postmortem interval, h</th>
<th>Decomposition&lt;sup&gt;b&lt;/sup&gt;</th>
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<td>Thermal injuries and smoke inhalation due to setting fire in a barricaded room</td>
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</tbody>
</table>

<sup>a</sup> A, accident; S, suicide.

<sup>b</sup> Decomposition of body apparent.
Fig. 1. Correlation of postmortem COHb concentrations as measured by CO-oximetry and UV spectrophotometry (Spec).

Deming regression analysis (A) and Bland–Altman bias plot (B).
Clinical Case Study

POINTS TO REMEMBER

1. Although COHb concentrations >50% are associated with death, deaths have also been seen at lower CO concentrations in cases of chronic exposure or the presence of comorbidities.

2. Interferences, such as MetHb, in the measurement of COHb in postmortem samples are a consideration, and proper steps should be taken to ensure the accuracy of CO-oximetry measurements when such interferences are present.

3. CO-oximetry, with the appropriate multiwavelength technology, can be a reliable and accurate method for postmortem COHb measurement.

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Commentary

Thomas G. Rosano

Olsen et al. report a fatality case in which postmortem measurement of carboxyhemoglobin was key evidence in a criminal prosecution. Although the increased carboxyhemoglobin concentration and the gross autopsy findings were consistent with carbon monoxide poisoning as a cause of death, questions of spectrophotometric method accuracy and reliability were raised by the defense.

In postmortem toxicology, spectrophotometry is an established and commonly used technique for measuring carboxyhemoglobin. The technique is based on Beer’s Law and takes advantage of the unique but overlapping wavelength spectra of blood oxyhemoglobin, deoxyhemoglobin, carboxyhemoglobin, and methemoglobin. Use of molar absorbivities at a number of selected wavelengths equal to or exceeding the number of hemoglobin forms allows the solution of a set of simultaneous equations and quantification of the hemoglobins. Although monitoring of at least 4 wavelengths is required, some current CO-oximeters monitor >100 wavelengths.

The analytical performance of the CO-oximeter is determined not only by the number of wavelengths monitored but also by applying sound forensic principles, including method validation. The authors of this case study show evidence of comparability of results...
for a CO-oximeter and manual spectrophotometric method and cite studies comparing results for postmortem blood carboxyhemoglobin measured by CO-oximetry and gas chromatography. It is important to recognize that CO-oximeters are developed primarily for clinical use. The effect of matrix interferences in postmortem blood (such as increased methemoglobin and variable total hemoglobin content) varies between CO-oximeters. In our experience, postmortem increases in methemoglobin can cause positive or negative carboxyhemoglobin interference, and pretreatment with sodium hydrosulfite can eliminate this effect. Validation of increased carboxyhemoglobin in medical examiner cases should be accompanied by confirmatory testing by a method with a different chemical principle. In forensic toxicology, confirmation testing is a recommended practice and adds to the reliability and forensic defensibility of toxicology findings.

Commentary

Barbara Jean Magnani

The key question when recovering a body from a fire is: Did this person die before or as a result of the fire? The corollary question is: If the person was dead before the fire, could the fire be a cover-up for murder? In the reported case, the answer to the main question was revealed during the autopsy. The fact that soot was found in the nose, mouth, trachea, and bronchi is incontrovertible evidence that the victim was ventilating (i.e., breathing) when the fire was started. That the defense would question the validity of the carboxyhemoglobin measurement is simply a way to obscure the facts and divert attention away from the true findings. That said, a carboxyhemoglobin concentration of 61% is consistent with the victim being alive before the fire was set. The laboratory results obtained must be interpreted in the context of the entire case, including the circumstances of death, the autopsy findings, and any relevant medical history.

A carboxyhemoglobin concentration >50% is generally accepted as evidence in itself to account for death; however, patients with medical conditions that compromise cardiac and/or respiratory function may succumb at much lower carboxyhemoglobin concentrations. With the advances in the technology, CO-oximetry is a reliable method for measuring carboxyhemoglobin and can provide accurate results with postmortem samples. The case described by Olson and colleagues provides corroborating evidence that newer instruments with multiple wavelength detectors are appropriate for measuring carboxyhemoglobin in postmortem samples, and results correlate well with those obtained with gas chromatography and ultraviolet spectrophotometry methodologies. In addition, forensic chemists should be aware of confounding factors (e.g., methemoglobin, sulfhemoglobin, low hemoglobin, or turbidity) when measuring carboxyhemoglobin in postmortem blood.

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