Original Contribution

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Left-right difference in carboxyhemoglobin saturation in heart blood of cadavers and its significance

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Objective: This study measured carboxyhemoglobin (COHb) in left and right atrial blood of cadavers and investigated the causes and significance of left-right differences in COHb.
Methods: After examining 216 cases in which COHb was detected from April 2006 through March 2017, we evaluated the correlation among the measurement results, particularly left-right differences in COHb and various other factors, including the autopsy findings and circumstances under which the bodies were discovered.
Results: Left-right differences in COHb ranged from -8% to 27%. Two cases of liver cirrhosis with splenomegaly had large left-right differences. Therefore, we investigated the spleen weight and left-right difference in COHb saturation, and found a correlation (r = 0.71), which might have occurred when blood stored in the spleen was released under hypoxia. Large left-right differences in COHb were also observed in individuals who were crushed by falling objects. This difference might have been caused by the obstructed venous return.
Conclusions: Multiple variations were found among the cases due to circumstances related to the deaths, making it difficult to draw clear conclusions about the causes of the left-right heart blood differences in COHb saturation. However, specific factors are apparently related either to the individuals or to the circumstances surrounding their deaths that caused significant left-right differences in COHb. Clarifying this may be helpful in estimating the process of death.

Key words: carbon monoxide poisoning, carboxyhemoglobin, splenomegaly

Introduction

Carbon monoxide (CO), a colorless and odorless gas, is generated when carbon or carbon compounds are burned in an insufficient oxygen supply, and in the exhaust of a motor vehicle. CO may be inhaled unknowingly, which regularly causes accidental deaths by poisoning. In Japan, CO is the leading cause of death by poisoning.[1] In actuality, CO poisoning is always possible whenever carbon is burned. CO can be a cause of occupational accidents at construction sites and factories, at homes when heaters are involved, at training schools, and even in restaurants. Proper ventilation is necessary to prevent such accidents.[2]

When CO is inhaled, it binds rapidly to hemoglobin (Hb) with more than 200 times greater affinity than that of oxygen. It produces carboxyhemoglobin (COHb), which causes tissue hypoxia. At low concentrations, CO causes nonspecific symptoms, such as headache, dizziness, nausea, and vomiting. Motor ataxia, a condition that can be difficult to diagnose, can occur if the presence of CO is not noticed in the body. At high concentrations of CO in the blood, people can become comatose; and respiratory or circulatory dysfunction can cause death.[2] People who die from CO poisoning exhibit bright-red livor mortis due to the absorption of COHb in the blood. Consequently, external findings might be suggestive of CO poisoning.[3]

Diagnosing CO poisoning requires verification of COHb presence in the blood. We measured COHb saturation in the left and right heart blood when CO was suspected to have played a role in the individual's death. In most cases, the left and right values were identical, but differences are not uncommon. In the present study, we

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investigated the causes and significance of differences in COHb saturation in the left and right heart blood.

Materials and Methods

Cases

COHb saturation in the blood was measured at the Department of Legal Medicine of Kitasato University School of Medicine for the 11-year period, from April 2006 through March 2017, in 216 cases where the circumstances indicated CO involvement. The Institutional Review Board for observation and epidemiological study, Kitasato University (B17-242), approved this study.

Specimen collection

Specimens were collected during postmortem examinations by piercing the body surface at the left and right margins of the sternum to collect blood from the left and right atria. After the blood samples were placed in sterile tubes, they were sealed immediately with a cap. In the autopsies, an incision was made in the pericardium to expose the heart. After the heart was removed, the descending vena cava was dissected. Then blood was collected from the right atrium. Subsequently, the blood was wiped away from the pericardial cavity while using pressure to block the blood flow from the right heart system. The pulmonary vein was dissected. Then the blood was collected from the left atrium. As in postmortem examinations, the blood samples were placed in sterile tubes and sealed immediately with caps.

Measurements

COHb saturation was measured using spectrophotometry.[4] The test sample was diluted with 0.1% Na_2CO_3 (sodium carbonate). After $Na_2S_2O_4$ (sodium dithionite) was added, the mixture was left to sit for 15 minutes. It was then placed in a spectrophotometry cuvette. Then absorbency of 538 nm and 555 nm wavelengths were measured immediately using a spectrophotometer (UVmini-1240; Shimadzu, Tokyo).

Statistical analysis

We evaluated the measurement results to correlate, particularly, left-right heart blood differences, various factors including postmortem/autopsy findings, and the circumstances under which the bodies were discovered. A spreadsheet program (Excel; Microsoft) was used for the analyses using *t*-tests and correlation analyses.

Results

Summary of cases in which COHb was detected

During the 11-year study period, we performed postmortem examinations or autopsies of 6,008 cases at our facility. Of those, the presence of COHb in the blood was suspected based on the circumstances of death, cadaver findings, or other factors, resulting in COHb being detected in 216 cases. Of those, 131 individuals died of acute CO poisoning. As shown in Table 1, death in 79 cases involved the burning of briquettes or other substances in a room, vehicle, or a poorly ventilated space. Exhaust emitted into a vehicle was the cause of death of 4 individuals. Records show that 12 corpses were discovered inside a burning car, 23 other corpses had been burned in buildings, and 83 other corpses were discovered at the sites of fires. The deaths of 15 individuals were attributed to other circumstances. The "other" category included cases that were difficult to classify, such as those involving the use of heaters or indoor electrical generators, clothing that caught fire at work, electric shock in unclear circumstances (with burn damage to the body in the area of the shock), or hanging in a closet with burn damage to the body. Table 1 shows COHb values from the left atrial blood. When samples could not be collected, measurements of the right atrial

Table 1. Case summary and distribution of COHb saturation

Circumstances	Male	Female	Total	Age (mean)	Distribution of COHb saturation (%)				
					0－20	21－40	41－60	61－80	81－100
Burning of briquettes, charcoal, etc. (in a room, bathroom, vehicle, etc.)	68	11	79	20－74 (43.8)	2	4	4	37	32
Exhaust inside a vehicle	4	0	4	55－63 (58.8)	0	0	1	2	1
Discovered inside burning vehicle	11	1	12	32－75 (58.7)	4	4	2	2	0
Death by burning (indoors, outdoors)	10	13	23	24－84 (55.3)	19	3	1	0	0
Discovered at site of fire	64	19	83	12－94 (63.5)	14	15	21	21	12
Other	13	2	15	31－94 (57.9)	8	1	4	1	1

blood or aortic blood were used. Although some deceased individuals had received treatment, the distribution of COHb saturation shows that most cases of suicide by burning briquettes or other substances in a poorly ventilated space exhibited high values of ≥60%, although all but 1 case of death by burning exhibited low values of ≤30%. Values for deceased individuals that were discovered at the sites of fires varied considerably.

Twenty cases were excluded because both the left and right heart blood was difficult to collect as a result of burn damage, postmortem changes, or other factors. In some cases, a specimen could be collected only from

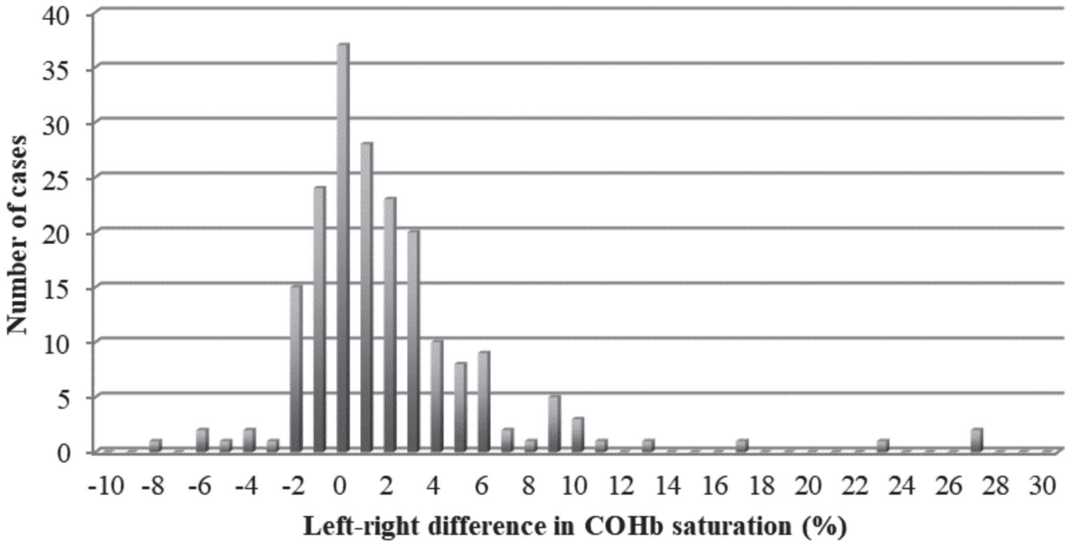

Figure 1. Frequency of left-right difference in COHb saturation

The most common COHb saturation measurement found had the same values in the left and right heart blood: a left-right difference of 0.

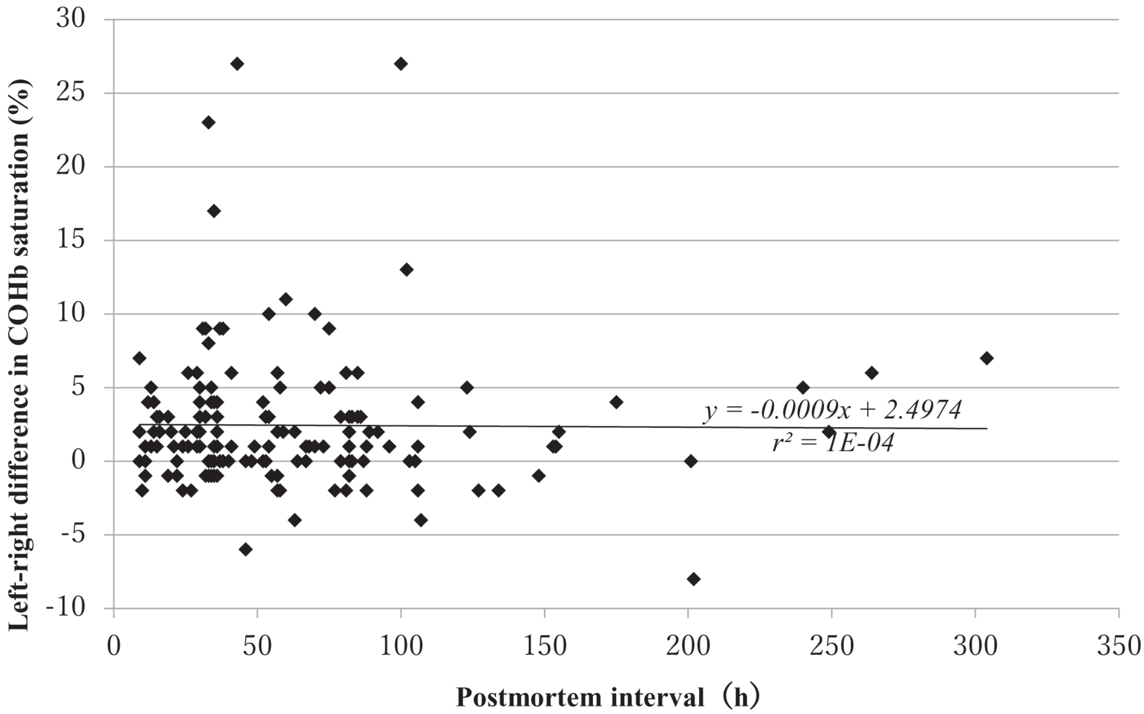

Figure 2. Left-right difference in COHb saturation and the postmortem interval

No clear correlation was found between the postmortem interval and the left-right difference in COHb saturation in the correlation analysis.

either the left or right atrium, or could not be collected from either atrium. Therefore, a sample from the aorta or elsewhere was measured. We were able to measure COHb saturation in both the left and right heart blood in 196 cases.

Differences in COHb saturation in the left and right heart blood

Subtracting the level of COHb saturation in the right heart blood from that in the left heart blood yielded differences ranging from -8% to 27%. Figure 1 shows the frequencies of these differences. A left-right

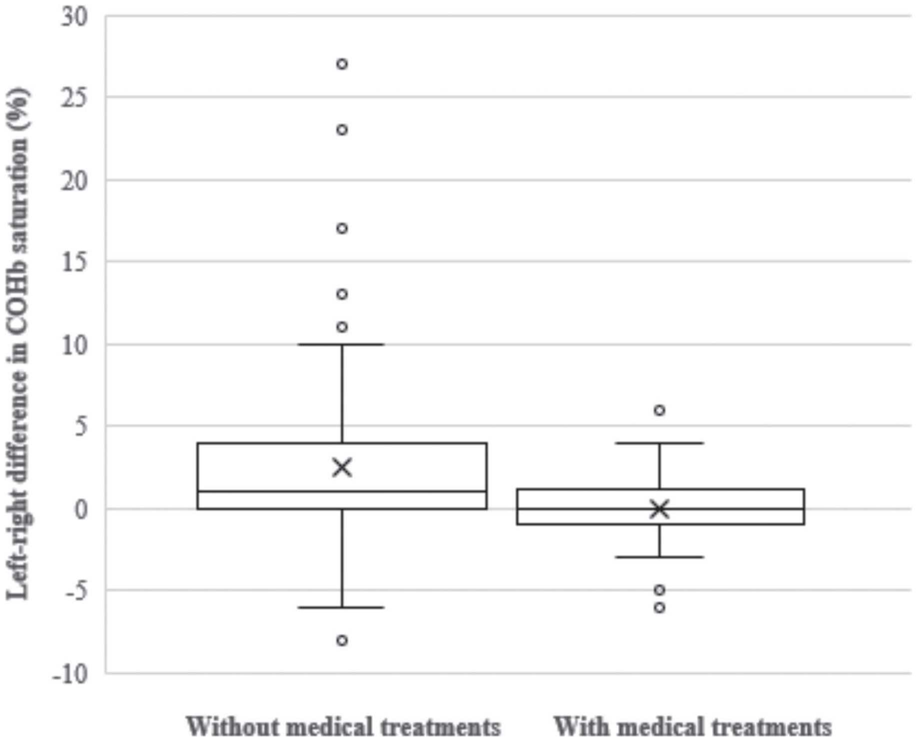

Figure 3. Left-right difference in COHb saturation in the cases with or without medical treatment

The value for cases without medical treatment was significantly larger. Wide variation was found in these cases.

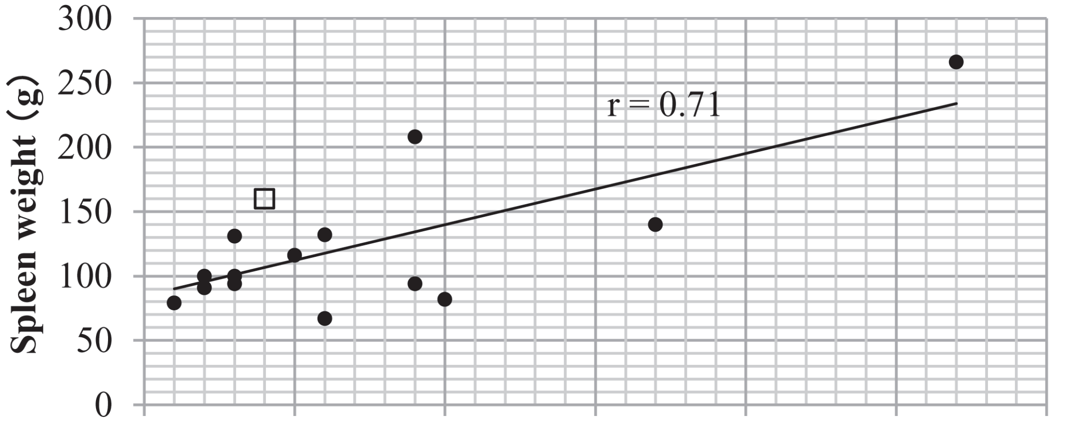

Figure 4. Left-right difference in COHb saturation and spleen weight

Left-right difference tended to increase as spleen weight rose in the correlation analysis.

difference of 0 was observed in the greatest number of cases (n = 37). Figure 2 shows the relation between left-right differences in COHb and the postmortem interval. We noted that no correlation was found between the postmortem interval and the left-right difference in COHb saturation. Figure 3 shows the distribution of left-right differences with or without treatment such as oxygen administration. The left-right differences in treated individuals were significantly smaller than those in non-treated individuals ($P < 0.001$). Because 2 cases of liver cirrhosis with splenomegaly had left-right differences, we investigated the relationship between the spleen weight and left-right difference in COHb saturation and found a statistical correlation of $r = 0.71$ (Figure 4). Large left-right differences in COHb were also observed in 3 individuals killed by falling objects (23%, 17%, and 23% left-right differences) (Data not shown).

There was an example of a corpse of a man in his 20s in which a ventricular septal defect was discovered (Figure 5). The spleen weighed 160 g, and the left and right heart blood COHb saturation percentages were 81% and 77%, respectively, represented as the square in Figure 4.

Discussion

The most poisoning deaths in Japan are attributable to CO.1 The number of suicides involving burning briquettes, or other substances, in poorly ventilated spaces is significant. Previous studies of COHb saturation have examined differences in measurements based on the collection site and the effects of disease, as well as investigated whether exposure to fire occurred before or after death based on the ratio of COHb in left and right heart blood.[5-8] However, to our knowledge, mechanisms or methods that provide accurate measurements in the left and right heart blood have not been sufficiently studied or reported in the literature.

Several reports have, however, described a postmortem phenomenon involving CO. Postmortem CO production has been observed in cadavers found in water.[9] The presence of COHb has been indicated by the formation of pink teeth because of postmortem changes.[10] We cannot rule out the effects of postmortem changes in the present study. Therefore, we examined the relation between the postmortem interval and left-right difference in COHb saturation, as shown in Figure 2. The concentration and duration of CO exposure differed in each case. Therefore, the initial values are not uniform. Although we cannot make any conclusions about how CO levels change over time, at least we did not observe a clear correlation with the left-right difference. No definitive conclusion can be made because of the small number of cases in the study. However, none of the cadavers for whom a relatively long time had elapsed after death exhibited large left-right differences. This lack of difference suggests that a state of equilibrium is approached gradually as time passes. Moreover, a decrease in the amount of left and right heart blood as time passes after death can cause the value to change. Therefore, to elucidate this, further investigations are warranted.

When air containing CO is inhaled, COHb forms in the lungs, then flows to the left atrium, left ventricle, and throughout the body, eventually reaching the right atrium and right ventricle, and is subsequently returned to the lungs. Therefore, under normal circumstances, it would be expected that saturation of COHb would be higher in the left heart blood than in the right heart blood.

In the present study, a number of cases exhibited higher COHb saturations in the left blood. However, we noted that some cases had higher COHb saturations in the right blood. Treatment might be one cause for this phenomenon. Some individuals who had died might have been administered oxygen or other substances during rescue procedures, albeit at different times and in different ways. Such procedures might have reduced the COHb saturation in the left ventricular side. Furthermore, in some pathological states, respiratory movements continue

Figure 5. Ventricular septal defect case

A 0.5-cm diameter defect was observed in the ventricular muscular septum. This case is denoted as (☐) in Figure 4.

even though the heart is not beating effectively (e.g., arrhythmia and cardiac arrest). This movement can increase COHb saturation in the pulmonary capillaries, which is then distributed to the right heart system via the pulmonary artery. In addition, when severe burns expose the chest cavity, CO can infiltrate directly through the venous walls.

Figure 3 shows the distributions of the left-right heart blood differences based on the presence or absence of treatment. The left-right differences among deceased individuals who had received treatment were significantly smaller than in non-treated individuals ($P < 0.001$). We believe that such treatment or removal from CO exposure caused COHb levels in the left atrial blood to decline. The left-right differences approached a state of equilibrium over time.

Whatever occurred, these factors do not act in isolation, but combine in various ways to affect the saturation of COHb in the left and right atrial blood. As previously noted, COHb saturation is generally expected to be higher in the left heart than the right heart blood. In this study, this difference was ≤27%. Large differences might result from the continuation of respiration in the states of either weak cardiac output or when death occurs quickly because of exposure to high concentrations of CO. However, our examination of cases with large differences revealed 2 cases involving liver cirrhosis that were discovered at the site of a fire (27% and 13% left-right differences) and 2 cases involving individuals who were killed by falling objects in the same fire (23% and 17% left-right differences). One case with a left-right difference of 27% was reported for reference purposes only due to an insufficient amount of specimen collected. Another case exhibited a difference of 11%, however nothing was remarkable about the circumstances related to that death.

Histologically, fibrous proliferation was observed in the Glisson's capsules of some cases, but liver cirrhosis was only found in 2 cases with large left-right differences in COHb. Both of these cases exhibited splenomegaly with spleen weights of 266 g and 320 g. The spleen stores blood in addition to its immune functions and those related to breaking down old erythrocytes. The spleen's blood storage capacity increases when splenomegaly is accompanied by conditions such as cirrhosis.[11,12] The spleen is known to contract during death by drowning, and hypoxia has also been reported to cause spleen contraction.[13,14] Although drowning is not equivalent to CO poisoning, elevated COHb saturation indicates that oxygen deprivation is occurring, which raises the possibility that the spleen is releasing erythrocytes. Splenomegaly in particular can contribute to raising the left-right difference in COHb because a large amount of blood is stored in the spleen. Therefore, we investigated spleen weight and the left-right difference in COHb saturation in autopsy cases; and as shown in Figure 4, there was a strong correlation found ($r = 0.71$). A link between greater spleen weight and larger left-right difference suggests that blood released from the spleen reduces COHb saturation in the right heart blood. However, although spleen contraction is highly likely in cases of drowning, in our experience, spleen contraction was infrequently observed in cases of CO poisoning. A study of the solid organs and COHb saturation in the blood revealed the strongest correlation with the spleen weight, compared to that of the liver, kidneys, or lungs,[15] which indicates that the spleen has a similar COHb saturation of erythrocytes in the blood. Therefore, something similar to displacement might occur in cases involving drowning, rather than a release of erythrocytes from another organ.

Only 15 cases would have been selected among those included in this study if we had selected the cases using the following three conditions. 1. The left or right heart blood is at least 60%, to ensure a sufficient amount of CO had been inhaled. 2. The specimen is at least 50 mL, to eliminate specimen-related error. 3. No medical treatment was administered, to eliminate any treatment effects. A limitation to this study is that it has a small number of cases; therefore, further studies are warranted with a larger study population. Additionally, blood mobilization has been observed in the tissue vascular beds of the kidneys, small intestine, and other areas in domestic rabbits during cerebral ischemia,[16] which suggests that organs other than the spleen should be studied to elucidate the pathological states in greater detail.

In addition to the 2 cases described above, in another case, the deceased was crushed by a falling object. The COHb saturation in both the left and right heart blood was 23% in that case, indicating that the person died quickly from burns. For the 2 cases described previously, the left and right values were, respectively, 95% and 72%, and 95% and 78%, indicating that death occurred because of acute CO poisoning. Although it is uncertain, because each of these individuals were crushed by a falling object, and even though what kind of object it was, and how they were crushed, remains unknown, it might have been possible that being crushed obstructed the blood's return to the right heart system, which increased the left-right difference. In addition, both were young, between the ages of 10 and 20, which is a time of robust hematopoiesis in the bone marrow. Consequently, the release of erythrocytes stored there might have played a role. However, the deceased individuals in both of these cases were discovered at the same fire in almost identical locations; therefore, the influence of other unique unknowable factors might also have played a role. Consequently, additional cases similar to these warrant examination.

A unique example exhibited a ventricular septal defect. A man in his 20s had a 0.5-cm-diameter defect in the muscular septum (Figure 5). Percentages for COHb saturation in left and right heart blood were 81% and 77%, respectively. His spleen weighed 160 g (the square in Figure 4). The left-right difference was small with respect to the spleen weight, which might have been because the defect allowed the left and right heart blood to mix.

Several reports in the literature have described studies of differences in the COHb saturation of the left and right heart blood. Nevertheless, these reports have only speculated vaguely that some kind of relation is likely to exist with the process of death. We also were unable to obtain a clear answer because of the unique circumstances related to each death, individual differences, and other factors. Nevertheless, we can report that the amount of blood stored in the spleen appears to contribute to raising the left-right difference (i.e., left > right). Moreover, factors that inhibit the return of blood to the right heart system, and the particular physiological functions of young people, might increase the left-right difference. Examining additional cases similar to these would help clarify the pathology of CO poisoning and the circumstances related to death.

Conflicts of Interest: None

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