Squeezed splenic blood sampling as an alternative method for carboxyhemoglobin measurement

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Abstract: Measurement of carboxyhemoglobin (CO-Hb) saturation in postmortem blood is important for diagnosing carbon monoxide poisoning or fire-related death. We discuss here the usefulness of squeezed splenic blood (SSB) as an alternative specimen for CO-Hb measurement. CO-Hb in SSB and femoral venous blood samples showed a good correlation. SSB appears suitable as an alternative specimen for CO-Hb measurement.

Key Words: carboxyhemoglobin; alternative sample; squeezed splenic blood.

Carbon monoxide (CO) is an odorless, colorless gas, and represents the leading cause of poisoning death in Japan [1, 2]. Measurement of carboxyhemoglobin (CO-Hb) saturation in blood is central to the diagnosis of CO poisoning or fire-related death [3]. The oximeter is widely used for CO-Hb measurement not only in clinical practice [4,5], but also in daily forensic practice [6-17]. Blood samples are essential for measuring CO-Hb [3], but cannot be obtained in forensic practice from bodies that are decomposed or have suffered severe blood loss. This study investigated the usefulness of squeezed splenic blood (SSB) as an alternative specimen for CO-Hb measurement.

MATERIALS AND METHODS

Femoral venous blood (FVB) and SSB were obtained at medicolegal autopsy from 36 victims (72 samples). FVB was collected by disposable pipette. SSB collection was performed using a disposable 1-ml syringe (SS-01T; Terumo, Tokyo, Japan) from the cut surface of the spleen at autopsy. Subjects comprised 26 males and 10 females (age range, 21-97 years), including 18 victims of CO poisoning or fire-related death. Causes of death for the other 18 victims were classified as follows: drowning, n=4; trauma, n=3; asphyxia, n=2; hypothermia, n=2; drug poisoning, n=2; and natural diseases, n=5. Post-mortem intervals ranged from approximately 9 h to 6 days (Table 1).

All measurement of CO-Hb from samples was performed using an AVOX4000 system (International Technidyne Corporation, Edison, NJ) at the time of autopsy. Operation of AVOX and the preparation of samples were performed in accordance with the specifications of the manufacturers [16-18]. In brief, approximately 50 µl of sample blood was filled into the cuvette and inserted into the AVOX4000 system, providing automatic analysis within 10 s. The operating range of CO-Hb is from 0% to 75%, with values over 75% shown as >75%. CO-Hb values were compared between
SSB and FVB.

Initially, simple regression analysis was used to measure the strength of associations between CO values from SSB and FVB. Statistical analysis was performed using the Mann-Whitney U-test. Values of p<0.05 were considered statistically significant. We also performed Bland-Altman analysis [19] to assess the level of agreement.

RESULTS AND DISCUSSION

CO-Hb concentrations from samples ranged from 0.0% to >75%. In 6 cases, CO-Hb concentrations of samples were >75%. As the operating range of the AVOX4000 system is 0-75%, statistical analyses were performed after removing the six samples with values >75%. The relationship between CO-Hb values from FVB and SSB is shown in Figure 1.

An excellent correlation was demonstrated between CO values from SSB and from FVB within the range of CO-Hb 0-75% (regression equation: \( Y = 0.841X + 0.521 \), where \( Y \) is CO-Hb in SSB and \( X \) is CO-Hb in FVB; correlation coefficient, \( r=0.961, n=30 \)). No significant difference was apparent in CO-Hb values from FVB and SSB (p=0.564). A Bland-Altman plot was generated to determine the level of agreement between FVB and SSB (Fig. 2).

The solid line represents the bias and broken lines represent the upper and lower 95% limits of agreements. FVB, femoral venous blood; SSB, squeezed splenic blood. The bias generated was 1.84%, and almost no discrepancy was observed between samples. Values in Cases 26 and 35 were outside of the range of the 95% limit of agreement. Cause of death in both cases was CO poisoning due to a house fire. The reason for this discrepancy may be that CO-Hb in blood had not reached a state of equilibrium at the time of death [20], although this remains unclear.

Evaluating the effects of CO in fire-related death or CO poisoning is important [3]. Blood is essential for CO-Hb measurement. Although the required sample volume for CO-Hb measurement is relatively small [3], blood is sometimes not readily obtainable due to the postmortem interval, postmortem degradation, or severe blood loss [21]. In the field of forensic science, alternative sources of specimens to blood for alcohol analysis, toxicological examination, or CO measurement have been described in previous reports [21-25]. Gas chromatography has been used for measurement of CO-Hb in tissue aliquots, as an alternative sample [24, 25]. The present study used an AVOX4000 portable oximeter, allowing easy handling with minimal sample preparation [16-18]. As the blood content in the spleen is relatively rich, SSB is usually easily collectable even in cases of severe blood loss. This may represent a useful alternative in cases where collection of a sufficient blood sample is difficult. Our results indicate that SSB is a valid option for CO-Hb measurement.

CONCLUSION

This study used SSB as a sample for CO-Hb measurement. A high correlation was identified between CO-Hb values from SSB and FVB, suggesting SSB as a useful alternative specimen for CO-Hb measurement.

Conflict of interest. The authors declare that they have no conflicts of interest.
References

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