High predictive value of red cell volume measurement using carboxy-haemoglobin in a rabbit model of haemorrhage

H. OBATA, F. GOTO, T. NARA, F. KUNIMOTO, N. ORIUCHI, E. MISHIBA AND M. NEMOTO

Summary
We have studied the accuracy of blood volume measurements using carbon monoxide (CO)-labelled haemoglobin (COHb) injection and dilution (CO method) by comparing changes in red cell volume (RCV) measured using the CO method and $^{51}$Cr-labelled erythrocyte dilution ($^{51}$Cr method) in a haemorrhage and infusion model in rabbits. RCV was measured repeatedly using the CO method at four different blood volume stages (stages I–IV). At stages I and IV, RCV was measured simultaneously using the $^{51}$Cr method. In comparing the sum of the circulating RCV and extracted RCV (SUM RCV) using the CO method, the values were almost equal and there were no significant differences between the values at the four stages. In comparing circulating RCV measured using the CO method and the $^{51}$Cr method, mean difference between the two methods was 0.80 (±0.76) ml kg$^{-1}$ or 4.7 (4.6)% and a positive correlation was observed ($r=0.91$). We conclude that the CO method can be used to measure blood volume during perioperative periods in infants because it avoids use of a radioactive tracer, is simple and repeated measurements are possible. (Br. J. Anaesth. 1998; 81: 940–944).

Keywords: measurement techniques, blood, volume; blood, volume; model, rabbit

It has been reported that haemodynamic data obtained from pulmonary artery catheters such as cardiac output and pulmonary capillary wedge pressure could not predict blood loss or erythrocyte deficit. The $^{51}$Cr-labelled erythrocyte dilution method ($^{51}$Cr method) for blood volume measurement has been reported previously. However, the use of radioactive tracer in the operating room or intensive care unit (ICU). Further, the CO method avoids use of a radioactive tracer and could be used to measure blood volume in children.

In this study, we have investigated the accuracy and reliability of the CO method in a haemorrhage and infu-
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anaesthesia and were then restrained in a standard stainless-steel rabbit holder in room air.

PROCEDURE FOR MEASUREMENT OF RCV

CO method

RCV was measured in four stages: normovolaemic stage (stage I); removal of blood (stage II); after infusion of physiological saline and hydroxyethylstarch (HES) (stage III); and after re-transfusion of the blood removed before stage II (stage IV).

At stage I, 4.5 ml of blood were obtained into a syringe containing heparin sodium solution. After measurement of haematocrit, 4 ml of blood were labelled by mixing with CO gas for 10 min in a 20-ml syringe. The mean COHb concentration in the CO-labelled blood was higher than 95% when mixing was completed, and remained at this concentration for more than 5 hr in the syringe. COHb concentrations were measured using a CO-oximeter (OSM3, Radiometer, Copenhagen, Denmark) adjusted in the animal mode for rabbit haemoglobin. Arterial blood-gas tensions were measured simultaneously (ABL300, Radiometer, Copenhagen, Denmark).

After obtaining two 0.5-ml samples of blood for measurement of baseline COHb concentration, 3 ml of CO-labelled blood were injected into the venous catheter. After injection of CO-labelled blood, two 0.5-ml samples of blood were obtained at 4, 8 and 12 min (fig. 1).

In our preliminary experiment, blood sampling for measurements of COHb concentration was performed at 2, 4, 8, 12, 16 and 32 min after injection of CO-labelled blood. However, the values at 2 min varied widely and were not considered for estimation of blood volume. The COHb regression line was stable for 4–12 min in rabbits (fig. 2) which has also been reported in dogs. Therefore, values at 4, 8 and 12 min were used for calculation of RCV in this study.

After injection of CO-labelled blood, several distinct dilutional phases are observed: an early circulation and intravascular mixing phase and a major dilution phase. In this study, a major dilution phase was achieved within 4 min, and its exponential decay was used for estimation of RCV circulating in the body. Therefore, the logarithms of this phase were extrapolated by the method of least squares to the time of injection of CO-labelled blood. Circulating RCV was calculated from the following equation:

\[
V_i(\text{ml}) \times H_t(\%) \times \text{COHb}_i(\%) = BV_{c}(\text{ml}) \\
\times H_t(\%) \times (\text{COHb}_i - \text{COHb}_b(\%)) \times (\%)
\]

where \(V_i\) = injected volume of CO-labelled blood; \(H_t\), \(\text{COHb}_i\), \(\text{haematocrit and COHb concentration in CO-labelled blood}\); \(H_t\), \(\text{haematocrit after injection of CO-labelled blood}\); \(H_b\), \(\text{haematocrit and COHb concentration in circulating blood before injection of CO-labelled blood}\); \(\text{COHb}_b\) = extrapolated COHb concentration in circulating blood at the time of injection of CO-labelled blood; and \(RCV_c\) and \(BV_c\) = circulating red cell volume and circulating blood volume, respectively.

The haematocrit of each sample was determined in duplicate using the micromethod. Capillary tubes were centrifuged for 5 min at 12 000 rpm. The difference

between peripheral and central haematocrit, which is termed an F cell ratio, influences calculation of blood volume. Whole-body haematocrit (Ht-b), obtained from direct measurement of RCV and plasma volume, is consistently lower than venous haematocrit (Ht-v). The ratio of Ht-b/Ht-v was reported to be approximately 0.9. Therefore, we used an F cell ratio of 0.9 in this study.

As shown in figure 1, after measurement at stage I, 13.5 ml of blood were obtained for CO-labelling which was used for measurements of blood volume at stages II, III and IV (4.5 ml for each measurement). In order to induce the hypovolaemic stage (stage II), another 22.6 ± 6.7 ml of arterial blood were then obtained, giving a total volume of blood removed of 12 ml kg\(^{-1}\). At stages II, III and IV, RCV was measured using the same method as that used at stage I. At stage III, RCV was measured after infusion of physiological saline 12 ml kg\(^{-1}\) and HES 12 ml kg\(^{-1}\).
At stage IV, RCV was measured after re-transfusion of the previously removed blood (22.6 ± 6.7 ml).

After each measurement, pure oxygen was administered via a face mask for 30 min to facilitate dissociation of CO from haemoglobin. Baseline COHb concentrations before injection of CO-labelled blood were less than 2.5% at each stage.

The ⁵¹Cr method

RCV was measured using the ⁵¹Cr method² in stages I and IV at the same time as measurements using the CO method. Initially, 2.5 ml of blood were obtained and labelled with ⁵¹Cr. After centrifugation and washing in physiological saline, ⁵¹Cr-labelled erythrocytes were suspended in physiological saline. Haematocrit and ⁵¹Cr radioactivity were measured with 0.5 ml of this suspension. After injection of 1 ml of the ⁵¹Cr-labelled erythrocyte suspension, 0.5 ml of blood were obtained at 10, 20 and 30 min for measurement of ⁵¹Cr radioactivity in circulating blood (fig. 1). Another 1 ml of ⁵¹Cr-labelled erythrocyte suspension was used for measurement at stage IV. At stage IV, before injection of ⁵¹Cr-labelled erythrocyte suspension, two 0.5-ml samples of blood were obtained for measurement of baseline ⁵¹Cr radioactivity in circulating blood as an indication of residual ⁵¹Cr radioactivity used at stage I.

In these measurements, haematocrit of all blood obtained was measured for calculation of SUM RCV and extracted RCV (RCVₑ). SUM RCV and RCVₑ were defined as follows.

\[
\text{SUM RCV} = \text{RCV}_c + \text{RCV}_e
\]

where RCVₑ = sum of the RCV removed to induce the hypovolaemic stage and RCV for labelling and sampling which existed extracorporeally at each measurement.

STATISTICAL ANALYSIS

Data are presented as mean (SD). One-way analysis of variance followed by Scheffé’s F test was performed to compare differences between individual measurement data. \(P<0.05\) was considered statistically significant.

Results

HAEMODYNAMIC OBSERVATIONS

Mean arterial pressure in stage I was 106 (12.5) mm Hg, which decreased significantly to 79 (15.0) mm Hg, 85.8 (14.8) mm Hg and 91 (11.5) mm Hg during stages II, III and IV, respectively.

CHANGES IN HAEMATOCRIT

Haematocrit before measurement of stage I was 41.8 (2.5)%. Haematocrit of CO-labelled blood for measurements of stages II, III and IV was 40.3 (2.6)%. Haematocrit before measurements during stages II, III and IV were 36.2 (1.4)%, 27.5 (2.2)% and 33.4 (1.7)%, respectively.

COHb and ⁵¹Cr concentrations

Mean COHb concentration of CO-labelled blood during all four stages was 97.0 (1.1)%. Figure 2 shows COHb concentrations of circulating blood in four measurements after injection of 3 ml of CO-labelled blood.

Using the ⁵¹Cr method, mean ⁵¹Cr radioactivity after injection of ⁵¹Cr-labelled erythrocyte suspension in circulating blood in stage IV (5786 (757) counts) was approximately 1.7 times higher than that in stage I (3341 (526) counts).

RED CELL VOLUME AND RELATIONSHIP BETWEEN THE TWO METHODS

Table 1 shows RCVₑ, RCV_c and SUM RCV at each measurement. In the CO method, there were no significant differences in the values of SUM RCV between the four stages. There were no significant differences between the values measured using the CO and ⁵¹Cr methods at stages I and IV. Figure 3 shows the correlation between the RCV_c values of the two methods and the regression line \((Y=0.99X+0.9, r=0.91)\). Figure 4 shows the difference in paired RCV_c measured using the two methods. The mean of the difference was 0.80 (0.76) ml kg⁻¹ and the mean of the percent difference was 4.7 (4.6)%, respectively.

After completion of the experiments, all rabbits were released from the rabbit holder and survived for more than 3 months without complication.

Discussion

In this study, we have demonstrated the accuracy of blood volume measurement using CO-labelled blood in rabbits. The values of SUM RCV measured using the CO method in all four stages were similar and a positive correlation \((r=0.91)\) (fig. 3) was observed between the values of RCV_c measured by the CO and ⁵¹Cr methods. The CO method overestimated 0.8 ml kg⁻¹ or 4.7% of the ⁵¹Cr method (fig. 4), indicating no clinically significant bias. In blood volume measurement, we do not know of any reports similar to ours which have compared the differences before and after haemorrhage using two different methods.

Previously studied blood volume measurements using CO were performed mainly by CO inhalation. Nomof and colleagues⁶ reported that RCV measured using the CO-rebreathing method was 16% larger.
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than that of the 51Cr method, as some CO was located in the extravascular space and some was excreted, metabolized or leaked. Root, Allen and Gregersen\(^9\) reported that RCV measured using COHb injection and dilution was 12% greater than that of the 51Cr method. One possible explanation for overestimation of the CO method is that a small amount of CO is released from haemoglobin and lost into plasma immediately after injection. Because in CO-labelled blood the plasma is saturated with CO, CO in plasma is diluted by circulating plasma and extracellular fluid when injected into the systemic circulation. The rapid decrease in COHb volume on injection may be responsible for overestimation of blood volume.

In this study, actual blood loss between stages I and II was 12 ml kg\(^{-1}\). However, loss of circulating blood volume calculated by the CO method was 10.2 (2.0) ml kg\(^{-1}\) (circulating blood volume values in stages I and II were 51.1 (3.7) ml kg\(^{-1}\) and 40.9 (2.3) ml kg\(^{-1}\), respectively). The difference in these values may be explained by the influx of extravascular fluid into the systemic circulation because the haematocrit at stage II decreased compared with that at stage I. Circulating blood volume is changed by extracellular fluid movement and infusion and therefore we compared the values of SUM RCV at four different blood volume stages for determining the accuracy of the CO method. If there was no unaccountable blood loss, SUM RCV should maintain at a constant value at each stage. However, the percent differences of SUM RCV between stage I and stages II, III and IV gradually increased and reached a maximum value of 6.0% at stage IV (the mean of the difference between stages I and IV was 1.2 (0.4) ml kg\(^{-1}\)). These small increases may be caused in part by unaccountable blood loss, such as bleeding from the surgical region, and blood loss during labelling and sampling.

In our preliminary experiment, we measured blood volume using the CO method without oxygen inhalation after each measurement. Baseline COHb concentrations in circulating blood at each stage increased gradually with repeated measurements. These increased baseline values generated a larger deviation in blood volume data and therefore we administered pure oxygen for 30 min after each measurement. Baseline COHb concentrations in the four measurements were within 0.4–2.4% in this study. These data suggest that oxygen inhalation is necessary before repeated measurements using the CO method. Pure oxygen inhalation rapidly decreased blood COHb concentrations. In contrast, \(^{51}\)Cr radioactivity in circulating blood in stage IV was approximately 1.7 times higher than that in stage I.

In a recent study, Fogh-Andersen and colleagues\(^{10}\) observed that the absorption spectrum of COHb, using an OSM3 analyser, depended on pH and \(P_{\text{CO}_2}\). To minimize this error, we included 5% CO in the calibration gas mixture for the two-point calibration.

<table>
<thead>
<tr>
<th>CO method</th>
<th>51Cr method</th>
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<tbody>
<tr>
<td>Stage I</td>
<td>Stage IV</td>
</tr>
<tr>
<td>Stage II</td>
<td>Stage III</td>
</tr>
<tr>
<td>RCV(_{\text{e}}) (ml kg(^{-1}))</td>
<td>19.1 (0.7)</td>
</tr>
<tr>
<td>RCV(_{\text{v}}) (ml kg(^{-1}))</td>
<td>0.37 (0.7)</td>
</tr>
<tr>
<td>SUM RCV (ml kg(^{-1}))</td>
<td>19.4 (0.7)</td>
</tr>
</tbody>
</table>

Table 1 Circulating red cell volume (RCV\(_{\text{e}}\)), extracted red cell volume (RCV\(_{\text{v}}\)) and sum of RCV\(_{\text{e}}\) and RCV\(_{\text{v}}\) (SUM RCV) in the four stages described in figure 1. Data are mean (SD). *\(P<0.05\) compared with stage I measured using the CO method.

Figure 3 Relationship between circulating red cell volume (RCV\(_{\text{e}}\)) measured using the CO and \(^{51}\)Cr methods. Each point represents RCV\(_{\text{e}}\) of six rabbits measured at stages I and IV, as noted in figure 1.

Figure 4 Difference in circulating red cell volume (RCV\(_{\text{e}}\)) measured using the CO and \(^{51}\)Cr methods plotted against their respective means. Each point represents RCV\(_{\text{e}}\) of six rabbits measured at stages I and IV, as noted in figure 1. The horizontal lines indicate mean (2 SD). The mean of the difference was 0.80 (SD 0.76) ml kg\(^{-1}\).
of the OSM3 analyser. $P_{aCO_2}$ in rabbits was maintained at 4.0–4.7 kPa throughout the experiment.

There are some disadvantages in the tracer dilution methods. It has been reported that the initial major dilution is achieved within 10–20 min in humans but is prolonged by circulatory dysfunction.8 In dogs, the major dilution phase was achieved within 10 min7 because the phase may be shorter than in humans because of smaller body size, smaller blood volume and higher heart rate. As it was shown that the dilution phase was achieved within 4 min in rabbits in our study, RCV was calculated from the data obtained at 4, 8 and 12 min after injection of CO-labelled blood.

Using our method, the toxicity of CO-labelled blood should be considered. COHb concentration in circulating blood was lower than 6% after injection of CO-labelled blood in all rabbits. A higher concentration of COHb is sometimes observed in smokers with no symptoms and is harmless in humans when the concentration is less than 10%.7,11,12 All rabbits in this study survived for more than 3 months without complications after completion of the experiments.

Several methods for blood volume measurement have been reported recently. Sugimoto and colleagues3 reported a method for continuous measurement of blood volume using $^{51}$Cr in rats. Throughout their experiment there was no change in erythrocyte volume and therefore changes in blood volume were calculated continuously from changes in the radioactivity of $^{51}$Cr in circulating blood. If there is bleeding, their method could not be used to calculate blood volume. Hoeft and colleagues4 reported a different method for measuring blood volume using ICG. However, blood ICG concentration is influenced by hepatic clearance, distribution of ICG, cardiac output and circulation time. A two-compartment model of the circulation is required for an adequate fit of the data. Abdominal surgery also changes splanchnic blood flow, and therefore measurement of blood volume using ICG may have some disadvantages during anaesthesia and surgery.13 Christensen, Rasmussen and Henneberg14 reported on the CO-rebreathing method using a to and fro system, and analysed CO kinetics by computer simulation. This method is useful for patients who are treated in the intensive care unit. They used a special system designed by Water’s Co. to avoid CO leakage. However, in the operating room it is difficult to use these types of instruments during surgery. In our method, special instruments and computer analysis were not necessary. Moreover, CO labelling, blood sampling, COHb measurements and blood volume calculation are completed within 30 min in the operating room. The CO method has advantages, especially for young patients, because it avoids the use of a radioactive tracer, and injection of CO-labelled blood was shown to be safe.

In summary, we have demonstrated the highly predictive value of RCV using a small volume of CO-labelled blood in rabbits. This method may be useful for repeated measurements of blood volume during the perioperative period in infants.

References


