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Circulating Blood Volume Measured by Pulse Dye-Densitometry

Comparison with ^{131}I-HSA Analysis

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Background: **Pulse dye-densitometry (PDD) is a newly developed technique for monitoring the arterial concentration of indocyanine green. Using this method, circulating blood volume (CBV) can be calculated without using radioisotopes. In this study, the CBV value obtained by PDD was validated by comparison using the human serum albumin (^{131}I-HSA) dilution method.**

Methods: **Eleven healthy volunteers underwent placement of cannulae into the radial artery and antecubital vein for withdrawal of blood samples and injection of indicator. Probes for PDD were attached to the right nostril and the right index finger. Indocyanine green (20 mg), dissolved in 4 ml water, and 25 μCi ^{131}I-HSA in 1 ml distilled water were injected simultaneously into the left antecubital vein. Blood samples were withdrawn 3, 6, 10, 20, 30, and 45 min after injection, then processed for spectrophotometric measurement of indocyanine green and scintillation counting.**

Results: **The blood dye concentration correlated well with the values obtained by PDD (r = 0.986, imprecision 0.04 ± 0.11 mg/l, 10.0 ± 31%. The imprecision of the CBV value obtained by PDD (nose probe) and by the ^{131}I-HSA dilution method was 3.99 ± 10.54%, 0.259 ± 0.593 l. The imprecision of the CBV obtained by *in vitro* spectrophotometry compared with PDD was 2.47 ± 9.00%, 0.100 ± 0.446 l.**

Conclusions: **This newly developed, less invasive method can measure CBV with an imprecision of 3.99 ± 10.54%, 0.259 ± 0.593 l (nose probe), and thus is also as accurate as the conventional radioisotope method (Key words: Blood volume determination; indocyanine green; iodine-radioisotopes; labelled blood; pulse spectrophotometry.)**

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PULSE dye-densitometry (PDD) is a newly developed technique that facilitates minimally invasive, subsequent measurement of cardiac output and circulating blood volume (CBV) by estimating the arterial concentration of indocyanine green (ICG) progressively. Studies have been undertaken to compare the derived cardiac output value obtained by the dye-dilution method and thermodilution,[1] or by thermodilution alone.[2] The clear advantage of this technique is that a pulmonary artery catheter is unnecessary.

Measurement of CBV may be useful for monitoring fluid distribution, especially in endotoxic shock[3] and other pathologic conditions.[4] Radioactive isotopes have been used to measure the CBV, but these methods are not suitable for routine bedside use. Several other different methods for estimation of CBV have been reported, including injection of carbon monoxide,[5] measurement of the cuff-occluded rate of increase in peripheral venous pressure,[6] or impedance.[7] However, these methods are difficult to perform and are not easy to apply clinically.

In the current study, we attempted to validate the CBV estimated by PDD by comparison with that obtained by the human serum albumin (^{131}I-HSA) method, which, up to now, has been one of the standard procedures for measuring CBV.

Materials and Methods

General Preparation

The experimental protocol was approved by the Research Committee of Kyorin University, School of Med-

icine. Eleven healthy adults (nine men, two women) were informed of the nature and risk of this study, and written consent was obtained. The subjects were administered a few drops of 5% Lugol's solution (prepared in Kyorin University Hospital) *per os*, 2 days before the start of the study, to prevent thyroid from accumulating ^{131}I-HSA. All experiments and measurements were performed in the radioactive management area of the Department of Radiology at Kyorin University.

A cannula was inserted into the left antecubital vein for injection of indicators, and a second cannula was inserted into the left radial artery for collection of blood samples. Blood pressure was measured intermittently with a cuff on the left arm. The subjects were asked to lie on a bed and rest until the hemodynamic parameters became stable. Probes were attached to the right nostril and the right index finger for PDD measurement. In a preliminary experiment, the nose probe was found to detect pulsation better than probes placed on the finger, lip, and ear. Therefore, we used a finger and a nose probe, and compared their clinical accuracy. Pulse dye-densitometry was performed using a DDG analyzer (Nihon Kohden Corp., Tokyo, Japan). The electrocardiogram and arterial oxygen saturation measured by pulse oximeter (Sp_{O_2}) also were monitored. Twenty-five microcuries of ^{131}I-HSA (Iodinated I 131 Albumin Injection; Dai-ichi Radioisotope Institute, Tokyo, Japan) was dissolved in 1 ml physiologic saline and 20 mg ICG (Diagnogreen; Dai-ichi Pharmaceutical, Tokyo, Japan) in 4 ml distilled water, and these were injected as a bolus followed by a flush of 20 ml of Ringer's acetate solution (Veen F®; Nikken Kagaku, Tokyo, Japan). Blood (3 ml) was withdrawn from the radial artery 3, 6, and 10 min after injection of ICG for determination of the arterial ICG concentration by spectrophotometry (U-2000; Hitachi Ltd., Tokyo, Japan) after centrifugation at 3,000 rpm at 4°C for 10 min. The ICG concentration was plotted semilogarithmically and back-extrapolated to the mean transit time point and the initial ICG concentration was obtained for calculation of CBV (fig.1). Ten milliliters of blood also was withdrawn for scintillation counting 10, 20, 30, and 45 min after injection. Samples were assayed for 3 min with a gamma scintillation counter (ARC-500; ALOKA, Tokyo, Japan). The background was obtained from a vacant tube because none of the volunteers had a history of intentional exposure to radioactive substances. Residual radioactivity in the syringe and three-way cock was assayed independently and subtracted from the administered radioactivity. The initial scintillation count diluted by CBV was calculated by back-extrapolation of the decay curve for the scintillation count at the injection time point. Circulating blood volume was calculated by dividing the administered radioactivity by the diluted radioactivity obtained from back-extrapolation. The hemoglobin concentration in each sample was determined using a hemoximeter (OSM3; Radiometer, Copenhagen, Denmark). The hematocrit concentration was measured by centrifugation in a glass capillary, and the value was used for calculation of blood-ICG concentration.

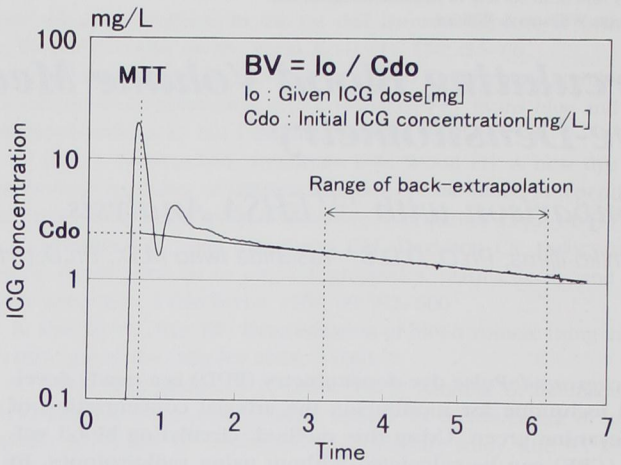

Fig. 1. Calculation of circulating blood volume (CBV). The CBV can be computed as: CBV (l) = I_0 (indocyanine green [ICG] dose administered)/Cd_0 (initial blood ICG concentration). Arterial ICG concentration decreases exponentially. The regression line between 2.5 and 5.5 min after mean transit time (MTT) was back-extrapolated to the time point of the MTT for the first circulation of ICG.

Pulse Dye-Densitometry

Pulse dye-densitometry is based on the principle of pulse spectrophotometry. Wavelengths of 805 and 890 nm are used for calculation of the blood-ICG concentration because the peak optical absorption by ICG occurs at 805 nm and is not significant at 890 nm. Absorption by oxyhemoglobin and deoxyhemoglobin is similar at 805 nm. The ratios of light absorption at 805 and 890 nm and the hemoglobin value were used to calculate the ICG concentration. The basic principle has been explained in detail elsewhere.[1] The optical probe essentially is the same as that used for pulse oximetry. Detection of arterial ICG is based on the fractional pulsatile change in optical absorption at 805 nm. The concentration of ICG (mg/l) is computed continuously, and a dye-densitogram is displayed on the computer screen. The frequency of subsequent measurement depends on how rapidly ICG is eliminated from the liver. Usually, the ICG

concentration in the blood becomes less than 1% of the initial value in 20 min, and therefore, subsequent measurement within a 20-min period is possible. However, if dye elimination by the liver is poor, this interval has to be prolonged.

Circulating blood volume was computed as CBV = I0/Cd0, where Cd0 is the concentration of dye (mg/l) computed by back-extrapolation of the clearance curve between 2.5 min and 5.5 min after the mean transit time (MTT) point of the first circulation to MTT (Fig. 1), and I0 is the amount of ICG administered (mg). The MTT point is assumed to coincide with the onset of dye elimination by the liver. Cardiac output is calculated from the area of the first circulation of ICG divided by I0. The PDD value has already been validated and reported.[1]

Statistical Analysis

Values are expressed as the mean ± SD. The degree of agreement between any two methods was determined by Bland-Altman analysis.[8] The difference between any of the values, CBV, and cardiac output was analyzed by paired *t* test. The Statview version 4.0 (Brain Power, Calabasas, CA) software package was used for the calculations.

Results

The mean weight and height of the 11 healthy volunteers were 60.9 ± 8.0 kg and 171 ± 7 cm, respectively. The blood dye concentration correlated well with the value obtained by PDD (nose): r = 0.986, and imprecision was 0.04 ± 0.11 mg/l, 10.0 ± 31%; n = 30 (Fig. 2) (all values including 10-min values), 0.05 ± 0.12 mg/l, 3.5 ± 10.5%; n = 20 (directly affecting values for calculation of CBV, 3 and 5 min after injection) PDD, finger: r = 0.887, imprecision = 0.20 ± 0.35 mg/l. The results for one subject were excluded from the analysis because the blood samples from this person were turbid even after centrifugation and could not be processed for spectrophotometric measurement of ICG. The correlation coefficients (r) for the CBV values measured by ^{131}I-HSA dilution, and those determined by PDD (nose; finger) were 0.887 and 0.853, respectively. The imprecision of the PDD method (nose; finger), compared with ^{131}I-HSA method was 3.99 ± 10.54%, 0.259 ± 0.593 l and 2.01 ± 17.89%, 0.496 ± 1.154 l, respectively (fig. 3). The correlation coefficients (r) for the CBV values measured by *in vitro* spectrophotometry and those obtained by PDD using the nose or finger probe were 0.943 and 0.960,

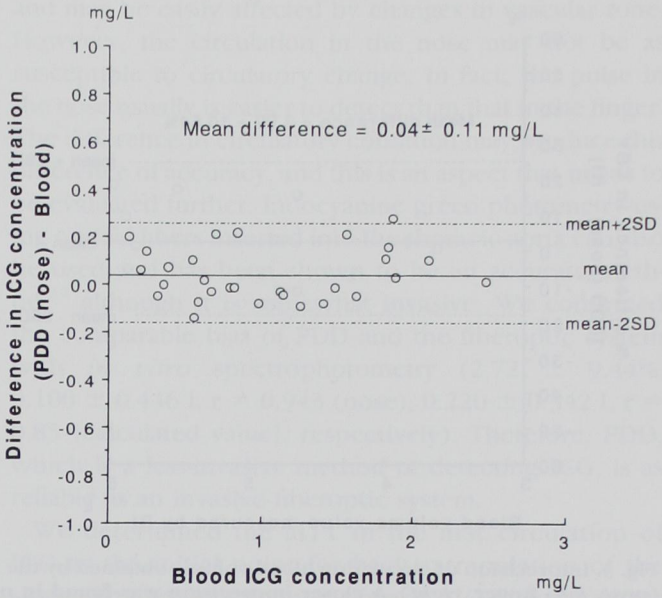

Fig. 2. Imprecision of indocyanine green concentration obtained by pulse dye-densitometry (nose) compared with *in vitro* spectrophotometry. Close agreement was obtained.

respectively. The imprecision of the CBV value obtained by *in vitro* spectrophotometry and that obtained by PDD (nose; finger) was 2.72 ± 9.44%, 0.100 ± 0.446 l, −0.342 ± 13.97%, and 0.321 ± 0.685 l, respectively (fig. 4). The bias of the CBV value between the nose probe and the finger probe was −0.98 ± 14.12%, 0.002 ± 0.85 l. The correlation coefficient between the CBV value obtained by *in vitro* spectrophotometry and the ^{131}I-HSA dilution method was 0.943. The bias of the CBV obtained by *in vitro* spectrophotometry and that obtained by ^{131}I-HSA dilution was 2.78 ± 13.86%, 0.233 ± 0.665 l (fig. 5). All these comparisons between two values are summarized in table 1.

The mean cardiac output was 5.26 ± 0.94 l/min (nose) and 5.95 ± 1.53 l/min (finger). The mean hematocrit value and hemoglobin concentration was 40.8 ± 3.6% and 14.3 ± 1.3 g/dl, respectively.

Discussion

Circulating blood volume has been measured previously using radioactive isotopes.[4] Although this approach yields useful data, it is not suitable for routine monitoring of CBV because the method is complex and carries the potential biohazard of a radioactive indicator. Indocyanine green, a nonradioactive substance, has been used as a dilution indicator in the measurement of

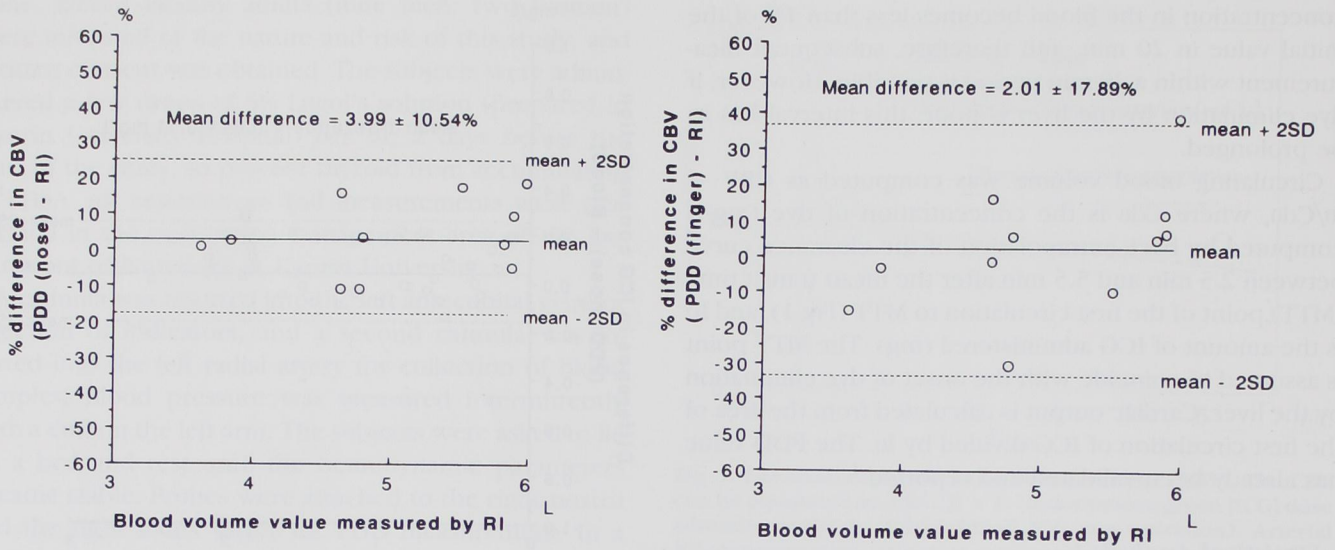

Fig. 3. Imprecision of circulating blood volume obtained by the ^{131}I-HSA method in comparison with pulse dye-densitometry (PDD) (nose, *left*; finger, *right*). A closer imprecision was found in nose than in finger. The imprecision is almost identical to the bias between the ^{131}I-HSA dilution method and *in vitro* spectrophotometry (Fig.5). RI = ^{131}I-HSA method.

CBV.[9–11] The concentration of ICG is measured in timed blood samples, which limits its clinical use. The current PDD method makes it possible to estimate arterial ICG concentration noninvasively.[1]

The ICG concentration measured by PDD had an imprecision of 0.04 mg/l compared with the value obtained by *in vitro* spectrophotometry. The initial ICG concentration would be approximately 4 mg/l for calculation of the CBV, if the amount of ICG injected is 20 mg and the subject's CBV is 5 l. This small bias (0.04 mg/l, 10%) and standard deviation (0.11 mg/l, 31%) permit sufficiently accurate calculation of CBV by PDD. Although the percentage difference, 10 ± 31%, seems to be large, this value is calculated from all values, including the 10-min value, which is not used for calculation of CBV. Such a low concentration at 10 min causes a large error and deviation. We use only the values at approximately 3 and 6 min for CBV calculation. Exclusion of 10-min values reduces imprecision to 3.5 ± 10.5%. This result is very similar to that of our previous study.[1] A larger dose of

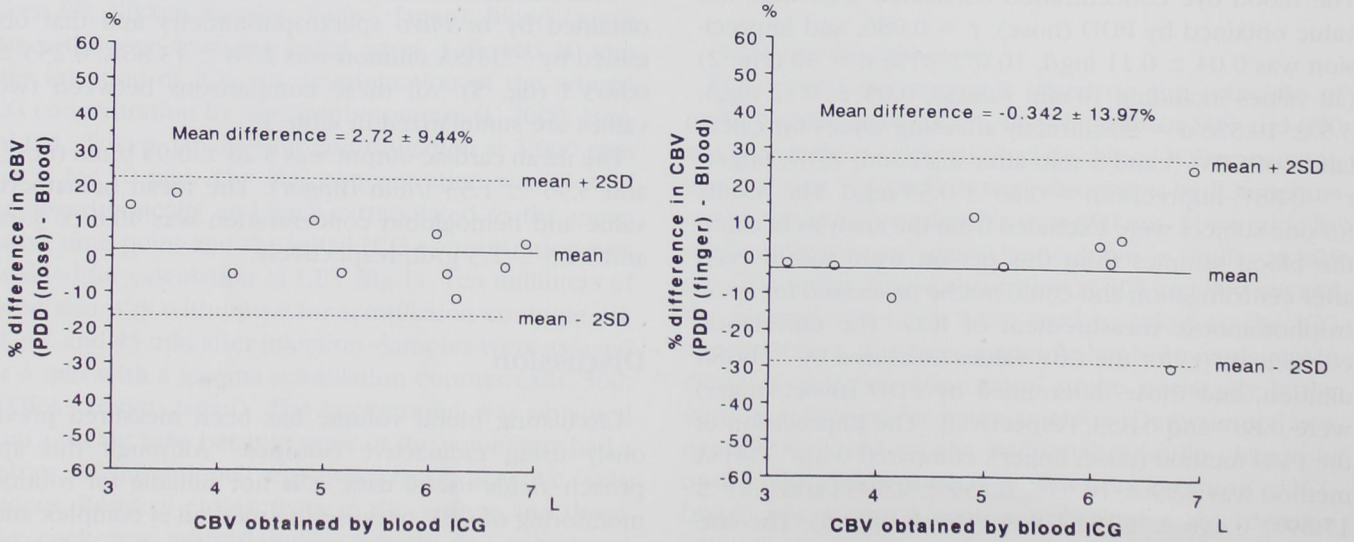

Fig. 4. Imprecision of circulating blood volume obtained by *in vitro* spectrophotometry compared with pulse dye-densitometry (nose, *left*; finger, *right*). The smallest imprecision in either method was observed for the nose probe value and the blood sample value. Blood = *in vitro* spectrophotometry

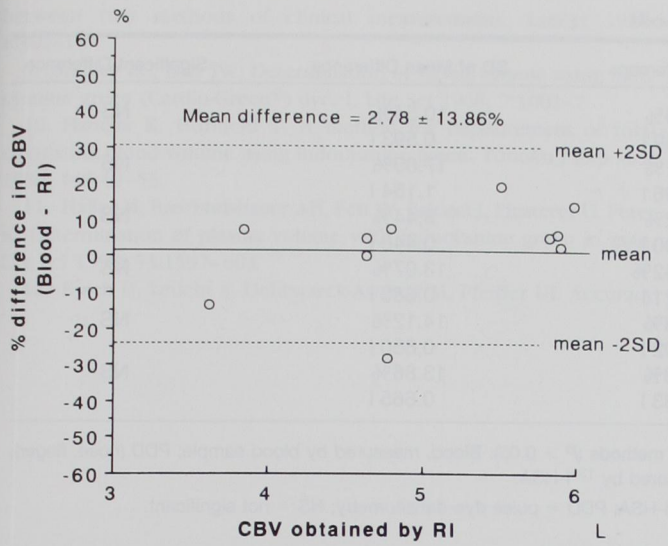

Fig.5 Comparison of the circulating blood volume value between the ^{131}I-HSA method and *in vitro* spectrophotometry. The bias between pulse dye-densitometry and ^{131}I-HSA (RI) method is almost identical. This level of bias may be attributable to the difference in the indicator used or the range of back-extrapolation.

injected ICG could prevent a low concentration from being included in the calculation, and thus would minimize error.

Only a few attempts have been made to compare different CBV measurements using tracers. Bradley and Barr[9] reported comparison of the CBV value obtained using "radioactive iodinated albumin" or ^{51}Cr, or both, with that obtained using ICG in six patients. The mean difference was −85 ± 372 ml, −1.8 ± 8.4% (n = 8 from six patients, calculated by the current authors from their data). Haneda and Horiuchi[10] also reported a comparison between the ICG method and the Evans blue method in dogs, and the mean difference was −41 ± 71 ml (mean CBV was 995 ml), −4.7 ± 6.7% (calculated from their data). The deviation of our data (3.99 ± 10.54%, 0.259 ± 0.593 l) was slightly higher than for their data. In view of the similar bias of *in vitro* spectrophotometry *versus* ^{131}I-HSA method (2.78 ± 13.86%, 0.233 ± 0.665 l) in this study, this slightly higher bias may be attributable not to PDD itself but to a different indicator.

The variation in the CBV value obtained by PDD using the finger probe was relatively large, approximately twice that obtained by PDD using the nose probe. Such a large variation also was observed in the ICG concentrations determined by *in vitro* spectrophotometry and PDD using the finger probe (0.20 ± 0.35 mg/l). Blood flow in the finger is remote from the central circulation and may be easily affected by changes in vascular tone. However, the circulation in the nose may not be as susceptible to circulatory change. In fact, the pulse in the nose usually is easier to detect than that in the finger. The difference in circulatory condition may produce this difference of accuracy, and this is an aspect that needs to be evaluated further. Indocyanine green photometry using optical fibers inserted into the thoracic aorta can also be used and has been shown to be an accurate method,[12] although it is somewhat invasive. We confirmed the comparable bias of PDD and the fiberoptic system with *in vitro* spectrophotometry (2.72 ± 9.44%, 0.100 ± 0.446 l, r = 0.943 (nose), 0.220 ± 0.342 l, r = 0.85 [calculated value], respectively). Therefore, PDD, which is a less-invasive method of detecting ICG, is as reliable as an invasive fiberoptic system.

We determined the MTT of the first circulation of ICG as the initial point for back-extrapolation of the slope. The half-elimination time of ICG is much faster (< 5 min) than radioisotope decay (^{131}I, 2–3 days), and hence the slope is steeper. Therefore, determination of the wrong time point for back-extrapolation might cause a large bias in the calculation. If the time is determined at the zero point, the CBV should be higher. The mean difference in the CBV value calculated by MTT from that calculated using radioisotopes is already positive. Therefore, the difference would become even larger if the zero time point were used for calculation. The MTT may be more appropriate for the calculation than the zero time point intercept. The back-extrapolation range was determined between 2.5 and 5.5 min after the MTT. In a series of preliminary experiments, the stable densitographic range was evaluated, and the period between 2.5 and 5.5 min was found to be most stable for extrapolation.

The decay of ICG is biexponential[13] and shows two different components: fast and slow. The CBV value obtained by our method, which analyzes the early component monoexponentially, corresponds to the fast component. Therefore, we call the value obtained by PDD the *circulating blood volume* (which has also been termed the *active blood volume* by Rothe *et al.*[3]) and not the total blood volume, which is calculated by biexponential analysis.[13]

One potential pitfall of this PDD method is the variation of the hematocrit value within the body. The hematocrit usually is obtained from a vein or a peripheral artery. In certain pathologic conditions, the difference in the hematocrit between the central and peripheral circulation becomes larger, and this ratio is called the F-cell

Table 1. Comparison of the CBV Value between Two Different Methods

	Correlation Coefficient (r)	Mean Difference	SD of Mean Difference	Significant Difference
RI *vs.* PDD (nose)	0.887	3.99% 0.259 l	10.54% 0.593 l	NS
RI *vs.* PDD (finger)	0.853	2.01% 0.496 l	17.89% 1.154 l	NS
Blood *vs.* PDD (nose)	0.943	2.72% 0.100 l	9.44% 0.446 l	NS
Blood *vs.* PDD (finger)	0.960	−0.342% 0.321 l	13.97% 0.685 l	NS
PDD (nose) *vs.* (finger)	0.958	−0.98% 0.002 l	14.12% 0.850 l	NS
Blood *vs.* RI	0.898	2.78% 0.233 l	13.86% 0.665 l	NS

There was no significant difference of the CBV values between any different two methods ($P > 0.05$). Blood, measured by blood sample; PDD (nose, finger), measured by pulse dye-densitometry using nose probe or finger probe; RI, measured by ^{131}I-HSA.

CBV = circulating blood volume; RI = circulating blood volume measured by ^{131}I-HSA; PDD = pulse dye-densitometry; NS = not significant.

ratio. For instance, hypovolemia creates a lower F-cell ratio when erythrocytes become heterogeneous within the body.[4] Such a difference should therefore be taken into account when estimating the CBV value. A varying F-cell ratio may produce a large error in the estimates.

Volume-deficit hypovolemia, seems to be a crucial factor for determination of prognosis in various clinical settings. Shoemaker *et al.*[14] reported that more than 50% of critically ill patients had a volume deficit of 0.5–2.0 l. Blood volume monitoring may help to identify such a volume deficit and act as a guide for fluid therapy. This current study was performed using healthy volunteers and did not evaluate whether this monitor was capable of following dynamic change. We evaluated CBV change before and after aortic clamping above the renal artery and observed a 27% change (unpublished data, 1996). It is expected that this monitoring system will make it possible to follow pathologic conditions. From our clinical experience, dynamic changes in the CBV value can be detected even during short periods of anesthesia. The CBV also may occasionally be affected by hemodynamic status and, therefore, may be a dynamic rather than a static parameter. Rothe *et al.*[3] reported that in an endotoxic shock model, the CBV (erythrocyte and plasma volume) decreased by nearly 30% after endotoxin infusion.[6] The CBV value may become a parameter that suggests a shift of intravascular blood to the extracellular space. We think that further accumulation of data using this monitoring system will reveal that the value of CBV is variable.

In conclusion, this newly developed, less-invasive method is capable of measuring CBV in human volunteers with a bias of 3.99 ± 10.54%, 0.259 ± 0.593 l (nose probe) and 2.01 ± 17.89%, 0.496 ± 1.154 l (finger probe) (mean ± SD) and is as accurate as the conventional radioisotope (^{131}I-HSA) method.

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