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Blood Volume Measurement at the Bedside Using ICG Pulse Spectrophotometry

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Background: **In the treatment of critically ill patients, blood volume (BV) measurement requires injection of some tracer substance and subsequent blood sampling to analyze the tracer concentration. To obviate both the sampling and laboratory analysis, techniques of pulse oximetry have been adapted to the noninvasive optical measurement in the patient's nose or finger of the arterial concentration of an injectable dye.**

Methods: **The authors report the clinical accuracy of a new noninvasive bedside BV measurement test that uses pulse spectrophotometry (the pulse method). The device detects pulsatile changes of tissue optical density of a nostril or a finger spanned by a probe emitting two infrared wavelengths (805 and 890 nm). After a peripheral or central intravenous injection of indocyanine green, the arterial dye concentration is continuously computed by reference to the previously measured blood hemoglobin concentration. Three types of tests of its accuracy are described here.**

Results: **In 10 healthy volunteers, the authors compared BV determined by the pulse method with an ^{131}I-labeled human serum albumin method. Three subject data sets were excluded because of motion artifact, a low signal:noise ratio, or both. For the other seven volunteers, the bias ± SD of pulse spectrophotometric BV values were 0.20 ± 0.24 l (or 4.2 ± 4.9%) for the nose probe and 0.34 ± 0.31 l (or 7.3 ± 6.9%) for the finger probe, with a mean BV of 5 l. In 30 patients who underwent cardiac surgery, the pulse method was compared with a standard indocyanine green method using intermittent blood sampling. In three patients, the BV could not be determined by the pulse method because of motion artifact, low signal:noise ratio, or both. In 27 patients, the bias ± SD of the BV by the pulse method was −0.23 ± 0.37 l (−5.3 ± 8.7%) for the nose and −0.25 ± 0.5 l (−4.2 ± 8.4%) for the finger. Patient BV ranged from 2.51 to 7.13 l (mean, 4.48 l). In 10 additional patients before cardiac surgery, BV was measured by the pulse method before and shortly after removal of 400 ml blood. The pulse method recorded a decrease of BV of 480 ± 114 ml. Three days after venesection, the mean BV was 117 ± 159 ml less than the predonation control.**

Conclusions: **In most patients, the pulse method provides bedside measurement of BV without blood sampling (except for hemoglobin determination), with an estimated error less than 10%. In 10–30% of tests the method failed because of motion distortion of the record during the 10-min data collection period or because of insufficient pulse amplitude in the test tissue. (Key words: Dye densitogram; effective hepatic blood flow; indicator dilution method; mean transit time; plasma disappearance rate.)**

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THE blood volume (BV) is one of most important factors that affect heart preload. Because directly measuring BV usually necessitates tracer injection, blood sampling, and tracer concentration analysis, adequacy of the volume of the patients' blood is clinically estimated in other ways, such as by monitoring central venous pressure or pulmonary capillary wedge pressure. Such estimates may be misleading because central venous pressure and pulmonary capillary wedge pressure depend not only on volume, but also on other factors, such as intrathoracic pressure, cardiac function, compliance of the veins, and posture.

The standard method for measuring BV has been indicator dilution[1] using a radioisotope[2] or Evans blue dye.[3] It has been difficult to measure BV serially because these

tracers are retained in the blood for days. Furthermore, minor risks are associated with the effects of the radioactive iodine and the mutagenicity of Evans blue dye.[4,5] In 1956, Fox and Brooker[6] introduced a new dye called indocyanine green (ICG). When injected into the blood stream, ICG rapidly binds to plasma proteins and is distributed in circulating BV. Because it is rapidly eliminated exclusively by the liver, it does not accumulate in the body[7] if the liver is intact and functioning. It has no known side effects other than a rare iodine allergy. Bradley and Barr[8] and other investigators reported[9-11] methods of BV measurement using ICG and indicated that it has good accuracy compared with other standard methods. The method has not, however, been widely adopted because it necessitates intermittent or continuous blood sampling to determine the time course of ICG concentration.

Pulse oximetry is a reliable, noninvasive method to measure arterial blood oxygen saturation.[12,13] A new modification called "pulse spectrophotometry" permits the clinician to measure continuously the ratio of the arterial concentrations of hemoglobin to ICG dye.[14] From this ratio and a measure of blood hemoglobin the circulating BV can be determined. Here we report the accuracy of this new method compared with standard laboratory techniques.

Materials and Methods

The Standard Radioisotope Method

The radioisotope method uses as a tracer ^{131}I-labeled HSA (Iodinated Human Serum Albumin; Daiichi RI, Tokyo, Japan). We injected a known quantity of ^{131}I-HSA (4-8 mCi) *via* an antecubital vein and drew blood samples 10, 15, and 20 min after injection. The indicator concentration was determined using a dilution computer system (Aloka, Tokyo, Japan), which consists of a scintillation counter and a computer. The mean BV computed from these three samples was used for comparison, with BVs estimated by the pulse method.

Blood Volume Using Indocyanine Green Measured Photometrically in Serial Blood Samples

A standard ICG method has been shown to correlate closely with the radioisotope method.[10] Mean transit time (MTT) cannot, however, be assessed in the intermittent blood sampling method; consequently, the method may produce some errors in BV calculation. These errors can be minimized by sampling blood from the pulmonary artery, accompanied by injection of ICG into the right atrium, because the MTT from the right atrium to the pulmonary artery is negligible. In patients with indwelling pulmonary artery catheters, we injected 10 mg ICG *via* a right atrial line. Four-milliliter pulmonary artery blood samples were taken immediately before the ICG injection and 3, 6, and 9 min after injection. After plasma separation, the plasma ICG concentration of each sample was measured using a spectrophotometer (U-2000; Hitachi, Ibaraki, Japan) using as a reference of a calibration curve that was prepared before. Because the distribution of ICG in blood is confined to the plasma,[7] ICG_{PL} concentration was converted to ICG_{WB} concentration using the following equation:

$$ICG_{WB} = \{(100 - Hct)/100\}ICG_{PL}$$

where ICG_{WB} = whole blood ICG concentration, ICG_{PL} = plasma ICG concentration, and Hct = hematocrit (%). The ICG concentration at injection time (Co), after mixing, was determined by semilogarithmic back extrapolation.

Noninvasive Measurement of Arterial Indocyanine Green Concentration

The pulse method applies the principles of pulse spectrophotometry to the measurement of the ratio of hemoglobin to arterial ICG concentration.[14] Hemoglobin is used as the reference light absorber because the concentration of hemoglobin in the blood is easily measured in clinical laboratories. This technique must be performed using a well-perfused tissue of the pulsatile optical density called a dye densitogram (DDG), from before intravenous injection of ICG until approximately about 10 min after injection. Wavelengths of 805 nm and 890 nm were selected because the extinction coefficients of oxygenated and reduced hemoglobin concentrations are nearly the same at these wavelengths (fig. 1). The peak absorption for ICG is 805 nm, whereas absorption of ICG at 890 nm is negligible. Light provided by light-emitting diodes is transmitted through a vascular bed (*e.g.*, a nostril or fingertip) to a photodetector positioned on the other side. Arterial pulsations of BV within the tissue bed change both the optical density at wavelengths absorbed by blood and to some extent the path length of light. The optical density, defined as incident light intensity/transmitted light intensity, is independent of the incident light intensity. The ratio of the variations caused by pulse (AC) to the total transmitted light (DC) at each of the two selected wavelengths depends empirically on the

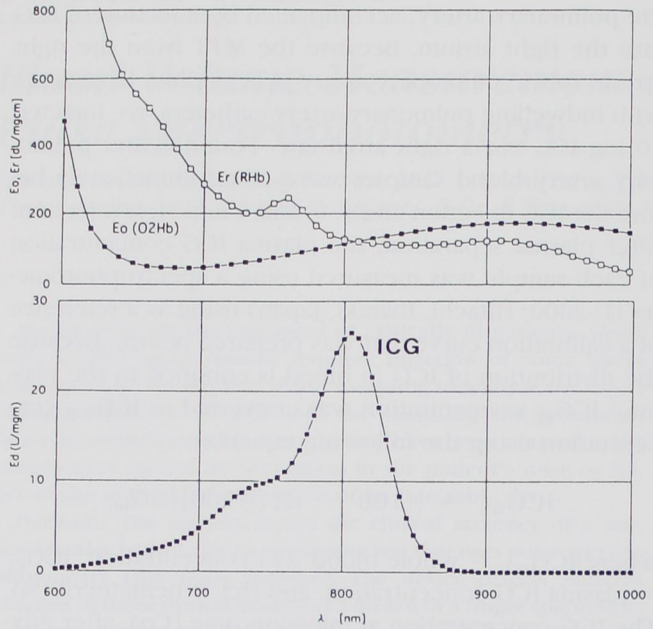

Fig. 1. Optical absorption characteristics of hemoglobin and indocyanine green (ICG). The absorption by hemoglobin is independent of oxygenation at 805 nm, isobestic wavelength, and reduced negligibly by desaturation at 890 nm. The peak absorption for ICG is 805 nm, whereas the absorption for ICG at 890 nm is negligible. The ICG pulse spectrophotometer uses the ratio of ratios of pulsatile to total light transmitted at these wavelengths to compute the ratio of arterial ICG concentration to arterial hemoglobin concentration. RHb = reduced hemoglobin; O_2 Hb = oxyhemoglobin; λ = wavelength; E = extinction coefficient (*i.e.*, absorbance). d = ICG dye.

ratio of arterial ICG concentration to arterial hemoglobin concentration.[14] From the ratio of ratios, AC:DC(805) to AC:DC(890), and the measured blood hemoglobin concentration, the actual concentration of ICG in arterial blood can be directly and continuously computed and displayed as a DDG.

Pulse Spectrophotometry Apparatus

A pulse spectrophotometry system has six major parts: a probe, a power source driving the light-emitting diodes, an analog amplifier, an analog-digital converter, a computer, and a printer. The probe has two light-emitting diode infrared sources, λ_1 = 805 and λ_2 = 890 nm, and a single photodiode detector. It is designed for easy attachment to a nostril or a finger. Pulse waves at λ_2, AC:DC ratios at both λ_1 and λ_2, and DDG are displayed in real time. After mixing of the injectate with all blood is complete, between 2.5 and 5.5 min after MTT, linear regression *versus* time of the logarithm of the DDG is used to calculate the clearance slope, from which the ICG concentration at MTT may be computed as Co. The instrument prints the early linear DDG, the entire semilog DDG and its linear regression line, and the calculated values of MTT, Co, and BV.

Dye Densitogram

Figures 2A and 2B show a typical DDG recorded from a nostril. Figure 2A is a linear plot of the first 2 min from which MTT can be determined using the modified Stewart-Hamilton technique.[15] The MTT[16] was calculated using the formula

$$MTT = \sum(Cn \cdot Dtn \cdot tn)/\sum(Cn \cdot Dtn)$$

where Cn = ICG concentration at nth time interval;

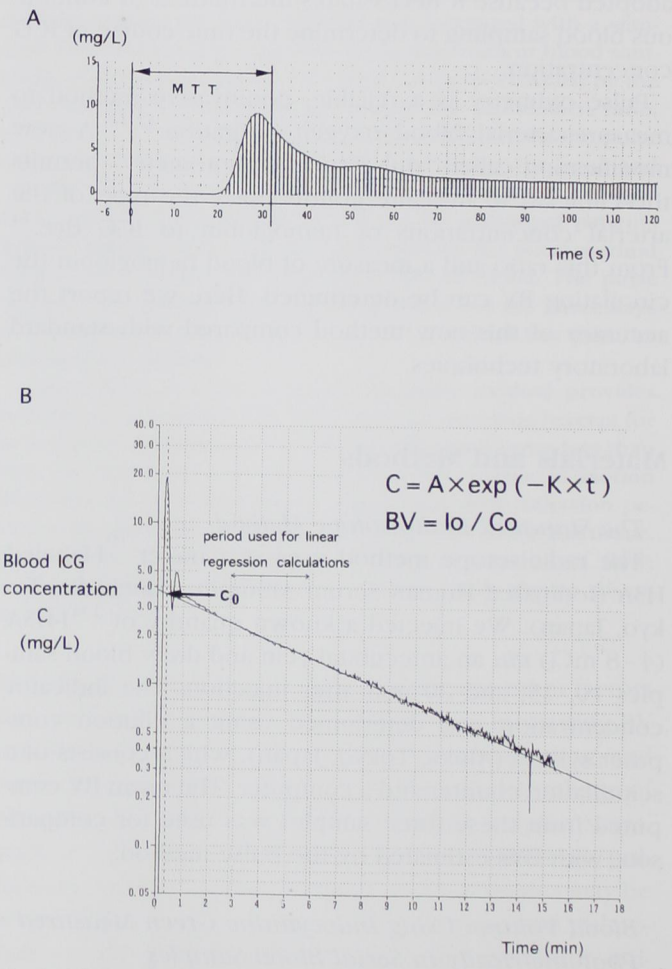

Fig. 2. (*A*) Initial 120 s of a dye densitogram (DDG). Vertical line at 32 s indicates mean transit time (MTT), computed as the center of gravity of the first circulation. This is considered as time zero of injection for determining the indocyanine green concentration at injection time (Co). (*B*) A 15-min semilog plot of a DDG. The clearance slope K (solid line) was computed by linear regression of semilog plot between 2.5 min and 5.5 min after MTT and extrapolated to MTT to compute Co.

Dtn = time interval (taken as 16 ms in this study); tn = time from the injection at which ICG concentration Cn is recorded; and n = 1, 2, 3 . . . . The ability of the method to measure MTT and dye concentration also permits an estimation of cardiac output.[14]

Figure 2B semilogarithmically displays dye concentration during the first 18 min. Because dye clearance is exponential, this plot permits back-extrapolation to MTT to determine the ICG concentration at injection time before clearance but after complete mixing (Co). Because the dye is cleared only by the liver, the technique may also be used to estimate liver blood flow.[14] Blood volume is then computed from Co and the injected dose of dye measured in milligrams (I): BV = I/Co.

Procedures

Using two instruments, probes were secured on a nostril and a finger. Before ICG injection, the pulse wave data were recorded with simultaneous monitoring of pulse oximeter saturation (SpO_2). After sampling blood to measure hemoglobin, we injected 20 mg ICG *via* an antecubital venous line or 10 mg ICG *via* a right atrial line while the DDG was recorded for 10 min. Indocyanine green causes a downward artifactual shift in SpO_2 readings.[17]

Data Analyses

The bias ± SD of the pulse method minus the standard methods was computed according to the method suggested by Bland and Altman.[18] To allow comparison with other methods to measure BV, which all used correlation coefficients and linear regression analysis to assess the degree of agreement, we calculated the correlation of the BV values determined by the two methods using linear regression (with the line forced to pass through points 0,0) and best-fit equations, although these have limited value in assessing agreement. Values are presented as the mean ± SD.

Results

Comparison with the Radioisotope Method

In 10 healthy volunteers, BV was measured by radioisotope and DDGs were recorded. Table 1 shows demographic data for the volunteers. Of these, the recorded DDGs of three nose probes and three finger probes were inadequate. In the seven healthy volunteers, compared with the radioisotope standard, the values of pulse method bias ± SD were +0.2 ± 0.24 l (4.2 ± 4.9%) with nose probes and +0.34 ± 0.31 l (7.3 ± 6.9%) with finger probes. The regression slope (constrained to 0 = 0) and correlation coefficient values were 1.04 and 0.94, respectively, for the nose and 1.07 and 0.91, respectively, for the finger.

Table 1. Demographic Data

	Normal Subjects	Surgical Patients	Blood Donor Patients
Gender (M/F)	10/0	14/16	6/4
Age (yr)	37 ± 11	55 ± 15	61 ± 12
Body weight (kg)	62 ± 6	57 ± 11	60 ± 11
Body height (cm)	168 ± 6	161 ± 12	163 ± 13
Hb (g/dl)	13.8 ± 0.7	11.1 ± 1.4	14.4 ± 1.8*

Values are mean ± SD.

Hb = blood hemoglobin concentration.

* Predonation value.

The Pulse Method versus *a Standard ICG Method in Patients Who Underwent Cardiac Surgery*

This part of the study was performed using an indwelling pulmonary artery catheter (Opticath; Abbott Laboratories, North Chicago, IL) in 30 patients after they underwent cardiovascular surgery. Informed consent was obtained from each patient before investigation. Table 1 shows demographic data for the patients. For both probes, noise or motion artifact prevented computation from the DDG of 3 of 30 patients. In 27 patients after cardiac surgery, assuming BV determined by ICG using sampled blood to be a standard, pulse method bias ± SD was −0.23 ± 0.37 l (−5.3 ± 8.7%) for the nose and −0.25 ± 0.5 l (−4.2 ± 9.4%) for the finger. Patient BVs ranged from 2.51 to 7.13 l (mean, 4.48 l). The regression slope (constrained to pass through the point 0,0) and correlation coefficient values were 0.95 and 0.94, respectively, for the nose and 0.93 and 0.99, respectively, for the finger.

Effect of Blood Donation on Blood Volume

The effects of blood donation on BV were studied in 10 patients who were scheduled to undergo elective cardiovascular surgery more than 4 days after blood donation (to provide for subsequent retransfusion if needed). For each patient, 400 ml whole blood was withdrawn *via* a cubital fossa vein. All patients provided informed consent to participate before investigation. Measurements were made after injection of 10 mg ICG *via* a forearm vein. The time course of BV was evaluated for each patient. Table 1 shows demographic data for this group of patients. Donation of 400 ml blood reduced the

BV value determined by the pulse method by 480 ± 114 ml. The corrected difference in BV between predonation and postdonation values, calculated as (BV_{POST} + 400 − BV_{PRE}), was −80 ± 114 ml (−1.4 ± 2.2%), ranging from −240 ml to 110 ml (−3.3 to 2.4%; difference not significant). Three days after donation, the estimated volume averaged 117 ± 159 ml (1.9 ± 2.2%) less than control (difference not significant).

Discussion

This article describes several tests of the accuracy of a pulse-spectrophotometry system that, with probes mounted on a finger or a nostril, permits noninvasive continuous measurement of arterial ICG concentration. The computer in the device subsequently calculates BV from the time course of arterial ICG concentration.

Several BV methods are used extensively despite limitations. The accuracy of all methods, including this pulse method, is limited by analytic precision, indicator leakage from the vasculature, or sequestration in tissue. The true values of BV are, therefore, beyond our knowledge. We can only compare the pulse method with other commonly used methods. For healthy volunteers, we chose one of the presumably more accurate standard methods using ^{131}I-labeled human serum albumin as an intravascular tracer. We compared a group of cardiac patients who already had pulmonary artery catheters to simultaneously use the same injection of ICG for both the pulse and the intermittent blood sampling methods. The pulse method agreed within approximately 10% with the reference methods in these tests. Agreement between commonly used methods to measure BV has been studied by several investigators. The bias of BV estimates was reported to be 0.3 ± 3.9% between the radiolabeled HSA method and the Evans Blue method in healthy volunteers,[19] 5.1 ± 2.3% between the ICG cuvette densitometry method and Evans Blue method in cardiac patients,[11] and 3.3 ± 4.2% between the ICG-sampled blood method and the ICG cuvette densitometry method in patients undergoing surgery.[10] The accuracy of the pulse method seems comparative to these BV methods. This study showed that BV estimated by the pulse method was 4–7% greater than that estimated by the ^{131}I-HSA method and was 4–5% less than by the ICG-sampled blood method. Thomsen *et al.*[19] found that BV estimates by the Evans Blue method were 0.3% greater than those by the radiolabeled HSA method. In cardiac patients, Haneda and Horiuchi[11] found that BV estimates by the ICG cuvette densitometry method were 5% greater than by the Evans Blue method. Haller *et al.*[10] reported that BV estimates by the ICG-sampled blood method were 3.3% greater than those by the ICG cuvette densitometry method. The results we obtained in the current study are consistent with those found in these investigations.

The pulse method was also used to detect changes in BV immediately after 400 ml blood was extracted by venesection in a group of patients donating blood before operation for their own use. The corrected difference in BV between pre- and postvenesection values was within 3.3% (difference not significant). The difference in BV between predonation and 3-day postdonation values, by which we could assess the reproducibility of the pulse method, was not significantly in error. The investigations of the reproducibility of BV methods revealed that the SD of the difference of the subsequent BV measurements was 6.5% in patients, using the radiolabeled HSA method[10]; 5.5% in dogs, using the Evans Blue method[20]; 4.3% in patients, using the ICG-sampled blood method[10]; and 5.1% in patients, using the ICG cuvette densitometry method.[10] The reproducibility of BV estimates by the pulse method was compared with these commonly used BV methods.

The assumption that at 890 nm the extinction coefficients of oxygen hemoglobin and reduced hemoglobin concentrations are the same causes BV to be underestimated. If oxygen saturation (Sa_{O_2}) ≥95%, the errors are estimated to be less than 10%. When Sa_{O_2} = 90%, the errors are estimated to be 20%. The smaller the Sa_{O_2}, the greater the errors. But all persons studied in this manuscript had Sa_{O_2} levels of more than 96%. Therefore, the assumption may not have serious effects on the results in this article. Perhaps the participants should inhale oxygen during BV estimation using the pulse method. After we completed this study, the manufacturer revised the algorithm to account for changes in oxygen saturation, with a resulting improvement in accuracy at low Sa_{O_2} levels.

In this study, we estimated BV ranging from 2.5 to 7.0 l. These results do not indicate the limits of accuracy of the measurement by the pulse method. Because the pulse method is based on measurements of arterial blood ICG concentration, adjusting the amount of ICG injected that is suitable to the patient's constitution can extend the limits of the measurement potential. In most cases, an injection of 0.2 mg/kg ICG may be adequate. Furthermore, the development of probes for children would

allow the estimation of smaller volumes of blood by the pulse method.

In the treatment of critically ill patients who may have rapidly changing BV, the pulse method may be especially useful in permitting subsequent measures within relatively short intervals (*e.g.*, every 30 min). Each indicator dilution method necessitates that the BV remain constant during measurement.[21] For the pulse method this is approximately 10 min. The delay before the next estimation depends on the patient's liver function. Patients with normal hepatic function (R15 < 5%, where R15 = residue percentile of ICG in blood 15 min after ICG injection) can clear 95% of injected ICG within 15 min, therefore, the estimation of BV by the pulse method can be repeated at 20-min intervals. Patients with hepatic failure (R15 > 35%), however, may take 1 h to clear 98% of plasma ICG.

If, because of motion, a probe position changes during data collection, an artifact is easily detected in the recorded AC:DC ratio. If pulse amplitude is inadequate, the recorded curve may be too noisy for analysis. In this study, we failed to obtain usable DDGs from either probe in 3 of 10 healthy volunteers and in 3 of 30 patients after cardiac surgery. We excluded these DDGs from the analysis. Two major causes underlie these failures. One was the distortion of DDG by body motion. Body motion is likely to dislodge probes, which alters the initial measurement conditions and resulting computation of the blood ICG:hemoglobin ratio, making it impossible to determine ICG concentration at injection time. The other problem was caused by insufficient circulation. When peripheral circulation is insufficient, the AC:DC ratios may be so low that the pulsatile signals are lost in the noise.

Figure 2B shows an appropriate DDG. Figure 3A shows a DDG that was inadequate because of dislodgement of the probe, which was apparent from the abrupt variation of the AC:DC ratio. An inadequate, noisy DDG resulting from a low AC:DC ratio is shown in figure 3B. Our experience suggests that the results are unreliable when the AC:DC ratio is less than 0.005 (0.5%). The AC:DC ratio varies according to the probe site. Generally, the face has a greater blood supply than the extremities. Consequently, the AC:DC ratio from a nose probe is likely to be larger than from a finger probe. We expect that ways of attaching probes to make them more resistant to body movement will be developed.

In existing methods for measuring BV using ICG, the concentration of ICG in the blood has been determined by repeated sampling from a peripheral vein after injection of ICG or by using a cuvette attached to a densitometer.[8,9,22] The value of Co depends on extrapolation to the time of injection (taken, when possible, as MTT).[11] In patients with poor circulation, MTT is often delayed, resulting in underestimation of BV. Using the pulse method, the dye density can be measured continuously, which allows accurate calculation of MTT. The cuvette densitometry method can also correctly estimate values for MTT, but that method has several drawbacks: (1) It

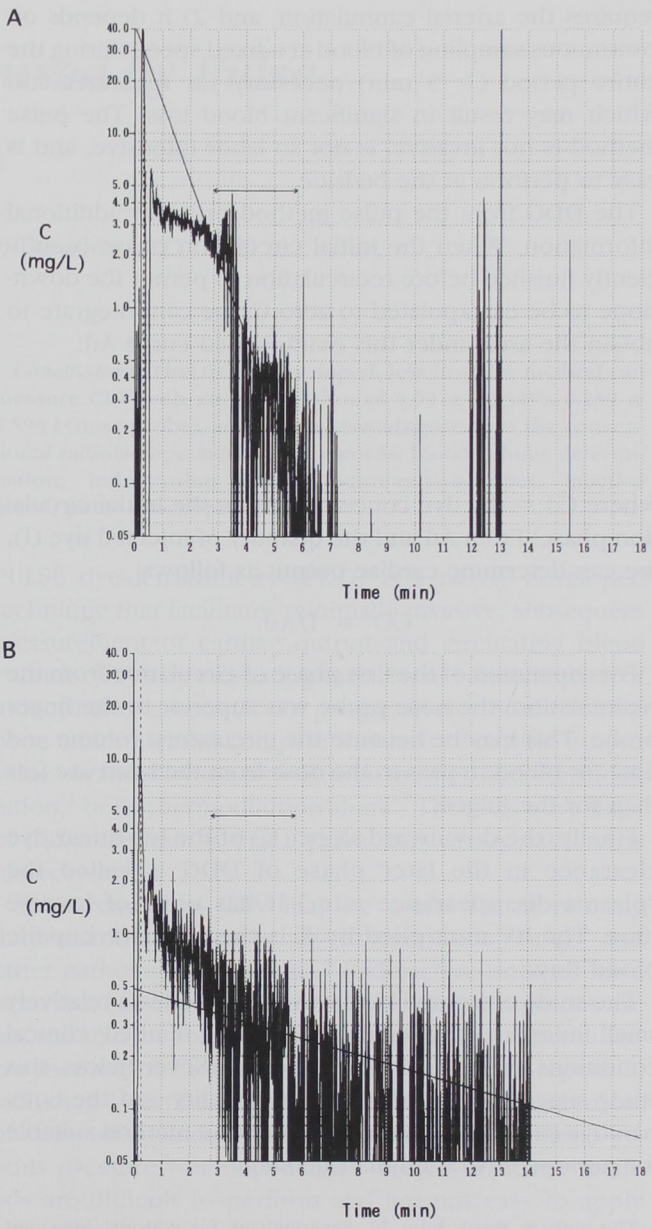

Fig. 3. Examples of defective dye densitograms that could not be analyzed. (*A*) Dislodgement of a probe. (*B*) A low ratio of the variations caused by pulse to the total transmitted light.

requires the arterial cannulation; and 2) it depends on continuous sampling of blood at a fixed speed during the entire period (> 5 min) necessary for measurement, which may result in significant blood loss. The pulse method is not invasive, is not so labor intensive, and is easy to perform at the bedside.

The DDG from the pulse method provides additional information. When the initial circulation phase is sufficiently finished before recirculation to permit the downslope to be extrapolated to zero C, we can integrate to obtain the area under this extrapolated curve Ad:

$$Ad = \int Cd.dt.$$

where Cd = the dye concentration in the initial circulation phase. From Ad and the quantity of injected dye (I), we can determine cardiac output as follows:

$$CO = I/Ad.$$

For separation of the first phase of circulation from the recirculation, the nose probe was superior to the finger probe. This may be because the circulatory volume and time for blood to pass to the nose from the heart are less than for the fingers.

Finally, the downward slope (K) of the log-linear dye clearance in the later phase of DDG is called the "plasma disappearance rate." It has units of inverse time. The BV multiplied by K is the effective hepatic blood flow.

The study presented here was performed in a relatively small number of persons and during limited clinical conditions. More studies are needed. Nevertheless, this study suggests that the multifunctionality and the comparative ease of use may make the pulse method suitable for perioperative fluid management.

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References

1. Yang SS, Bentivoglio LG, Maranhao V, Goldberg H: Blood volume, From Cardiac Catheterization Data to Hemodynamic Parameters. 3rd edition. Philadelphia, FA Davis, 1988, pp 114–21

2. Albert SN: Blood Volume and Extracellular Fluid Volume. 2nd edition. Springfield, Charles C Thomas, 1971, pp 161–7

3. Gibson JG, Peacock WC, Seligman AM, Sack T: Circulating red cell volume measured simultaneously by radioactive iron and dye method. J Clin Invest 1946; 25:838–47

4. Kornbrust DJ, Barfknecht TR: Comparison of 7 azo dyes and their azo reduction products in the rat and hamster hepatocyte primary culture/DNA-repair assays. Mutat Res 1984; 136:255–66

5. Nakane H, Balzarini J, De Clercq E, Ono K: Differential inhibition of various deoxyribonucleic acid polymerases by Evans blue and aurintricarboxylic acid. Eur J Biochem 1988; 177:91–6

6. Fox IJ, Brooker LGS, Heseltime DW, Wood EH: A new dye for continuous recording of dilution curves in whole blood independent of variations in blood oxygen saturation. Circulation 1956; 14:937–8

7. Cherrick GR, Stein SW, Leevy CM, Davidson CS: Indocyanine green: Observation on its physical properties, plasma decay, and hepatic extraction. J Clin Invest 1960; 39:592–600

8. Bradley EC, Barr JW: Determination of blood volume using indocyanine green dye. Life Sci 1968; 7:1001–7

9. Busse MW, Zisowsky S, Henschen S, Panning B, Reilmann L: Determination of circulating blood volume by measurement of indocyanine green dye in hemolysate: A preliminary study. Life Sci 1990; 46:647–52

10. Haller M, Akbulut C, Brechtelsbauer H, Fett W, Briegel J, Finsterer U, Peter K: Determination of plasma volume with indocyanine green in man. Life Sci 1993; 53:1597–604

11. Haneda K, Horiuchi T: A method for measurement of total circulating blood volume using indocyanine green. Tohoku J Exp Med 1986; 148:49–56

12. McCarthy K, Decker MJ, Strohl KP, Stoller JK: Pulse oximetry, Monitoring in Respiratory Care. Edited by Kacmarek RM, Hess D, Stoller JK. St Louis, Mosby, 1993, pp 309–7

13. Severinghaus JW, Honda Y: History of blood gas analysis. VII. Pulse oximetry. J Clin Monit 1987; 3:135–8

14. Iijima T, Aoyagi T, Iwao Y, Masuda J, Fuse M, Kobayashi N, Sankawa H: Cardiac output and circulating blood volume analysis by pulse dye-densitometry. J Clin Monit 1997; 13:81–9

15. Yang SS, Bentivoglio LG, Maranhao V, Goldberg H: Cardiac output, From Cardiac Catheterization Data to Hemodynamic Parameters. 3rd edition. Philadelphia, FA Davis, 1988, pp 47–58

16. Yang SS, Bentivoglio LG, Maranhao V, Goldberg H: Blood volume, From Cardiac Catheterization Data to Hemodynamic Parameters. 3rd edition. Philadelphia, FA Davis, 1988, pp 116–7

17. Scheller MS, Unger RJ, Kelner MJ: Effects of intravenously administered dyes on pulse oximetry readings. ANESTHESIOLOGY 1986; 65:550–2

18. Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986; i:307–10

19. Thomsen JK, Fogh-Andersen N, Bulow K, Devantier A: Blood and plasma volumes determined by carbon monoxide gas, ^{99m}Tc-labelled erythrocytes, ^{125}I-albumin and the T 1824 technique. Scan J Clin Lab Invest 1991; 51:185–90

20. Kisch H, Leucht S, Lichtwarck-Aschoff M, Pfeiffer UJ: Accuracy and reproducibility of the measurement of actively circulating blood volume with an integrated fiberoptic monitoring system. Crit Care Med 1995; 23:885–93

21. Lassen NA, Henriksen O, Sejrsen P: Indicator methods for measurement of organ and tissue blood flow, Handbook of Physiology, section 2, volume III. Edited by Shepherd JT, Abbound FM. Bethesda, American Physiology Society, 1983, pp 21–8

22. Wolthuis RA, Overbeck HW, Collings WD: Measurement of blood flow in the limb of man by cuvette densitometry. J Appl Physiol 1969; 26:215–20