Abstract. Hemodynamic stability during hemodialysis depends largely on plasma volume (PV) preservation during ultrafiltration (UF). Current estimates of blood volume (BV) are indirect or involve the use of radioactive tracers, which does not allow repeated measurements during hemodialysis. Indocyanine green was used to measure PV during hemodialysis. After an initial pilot phase (phase I), PV values were determined before dialysis, repeatedly during isovolumic hemodialysis (phase II), and during stepwise UF (phase III). Absolute BV values were calculated from PV and hematocrit values. Patients were monitored for extracellular fluid volume (bioimpedance monitoring) and relative BV changes (ultrasonic monitoring). Phase I demonstrated dye stability in plasma, peak absorbance at 805 nm, and a short half-life (4.53 ± 1.5 min). Ten milligrams of dye (2.5 mg/ml) were injected for each PV measurement. Eight plasma samples were obtained beginning 3 min after injection, at 1-min intervals, for assessment of decay characteristics. The isovolumic hemodialysis PV measurements demonstrated excellent reproducibility ($r^2 = 0.98$; method SD, 356 ml; mean coefficient of variation, 4.07%) and a difference of only 149 ± 341 ml (mean ± SD), compared with predialysis PV values (Bland-Altman method). PV values at the beginning of dialysis were significantly correlated with body surface area ($r^2 = 0.82$, $P < 0.001$) and extracellular fluid estimates ($r^2 = 0.73$, $P < 0.001$). BV prediction formulae significantly underestimated absolute BV at the start of dialysis ($P < 0.0001$). The findings demonstrate that this method can be used for repeated PV determinations during hemodialysis, with excellent reproducibility. It is a potential tool for further research on hemodynamic stability during UF.

Hypovolemia plays an important role in symptomatic hypotension, which complicates up to 25% of dialysis treatments. This has generated considerable interest in blood volume (BV) determinations during ultrafiltration (UF). Attempts to quantify absolute BV during hemodialysis have been limited by the lack of a suitable method. Methods involving radioactive tracers are unsuitable for routine clinical use and do not allow repeated measurements (1). In vitro studies have revealed a possible mutagenic potential for Evans blue (2). Therefore, current estimates of BV during dialysis are indirect or denote only relative changes and have been validated only with anthropometric data derived from the normal population (3) or with single predialysis measurements. In 1968, Bradley and Barr (4) reported on BV measurements with indocyanine green (ICG) for a limited number of patients. This method has since been used for liver blood flow estimations and cardiac output measurements but has not been studied during hemodialysis. The in vivo properties of this tricarbocyanine dye allow its repeated use within short periods (5). We therefore examined the feasibility of using the dye for repeated plasma volume (PV) determinations during hemodialysis. We wished to establish the technique for PV determinations during hemodialysis, using arteriovenous fistulae, and to determine the reproducibility of the technique, observe PV changes during dialysis, and compare BV findings with results from standard prediction formulae. In this report, we summarize our experiences with this method and present some results of its practical application.

Materials and Methods

Study Design

The study was performed in three phases.

Phase I (Five Studies)

We used this pilot phase to establish the method, dose, dye kinetics, and sampling and calibration techniques for hemodialysis patients. Dialysate samples were collected from the dialyzer outlet port for assessment of ICG absorbance immediately and 3 min after dye injection, for detection of any leakage of dye across the dialyzer.

Phase II (Nine Studies)

The purpose of this phase was to test reproducibility and method variations. PV was measured with the patient supine for 20 min just before dialysis (via fistula needles), followed by triplicate measurements (via the sample port) at 20-min intervals during an isovolumic period in the first 1 h of dialysis.

Phase III (10 Studies)

PV was determined directly during dialysis with UF (3 liters/h), with four UF steps (removing 40, 20, 20, and 20% of total UF volume)
and intervening rest periods; BV measurements were recorded at the beginning and the end of the first and fourth UF boluses, under steady-state conditions (Figure 1). All measurements and UF commenced after an equilibration period of 20 min after connection to the extracorporeal circuit.

Subjects
The North Herts ethical review committee approved the study. All patients gave informed consent. Twenty-four studies were performed during routine dialysis sessions for 17 subjects (including four female subjects) of different body sizes, with a wide range of interdialytic weight gains (Table 1). Subjects had been undergoing chronic hemodialysis for at least 6 mo, with stable dry weights and normal liver function test findings. Exclusion criteria included iodine allergy, eosinophilia, increased serum IgE levels, and significant access recirculation within 1 mo before the study. All patients were undergoing thrice-weekly high-flux dialysis (4008E dialyzer; Fresenius Medical Care), with polyamide membranes and arteriovenous fistulae. Blood flow rates were in the range of 350 to 450 ml/min, and the mean weekly Kt/V was 1.24 ± 0.16 (6).

Tracer
The tracer used was ICG (Cardiogreen; Fluka, Buchs, Switzerland), a tricarbocyanine dye (M, 775) with an absorption peak at 805 nm. The dye is nontoxic, confined to plasma, not subject to extravascular distribution, and not metabolized or degraded. After injection, the dye is rapidly bound to plasma proteins. After equilibration, the dye decays quickly, in an exponential manner. The dye is exclusively taken up by the liver and has a plasma half-life of 2 to 3 min (the time decayed rapidly, in an exponential manner. The dye is exclusively taken up by the liver and has a plasma half-life of 2 to 3 min (the time required for the initial concentration of the dye to be decreased by one-half) (7). The elimination characteristics resemble Michaelis-Menten kinetics (5).

Calibration
Calibration was performed for each BV measurement, with a blank plasma sample, just before dye injection. For five-point calibration, 30 ml of distilled water was added to 1 ml of neat ICG solution (2.5 mg/ml) to yield a dilute calibration fluid. Syringes were weighed before and after the contents had been added, for determination of exact volumes. A microcuvette was placed in the spectrophotometer (Phillips 8700 series) and zeroed at a fixed wavelength of 805 nm. A 1-ml baseline plasma sample was injected into the microcuvette, which was reweighed to establish the exact PV. The absorbance of the blank plasma sample was then determined.

Four 10-μl aliquots (10-μl micropipettes; coefficient of variation, <0.5%) of dilute calibration fluid were incrementally added to the plasma in the cuvette, and the absorbance was measured at each step. The mean of four readings was obtained at each step, and the cuvette was removed, agitated, and replaced between measurements. The dilution effect of addition of the aliquot volumes was taken into account, to improve precision.

For two-point calibration, a concentrated calibration fluid was prepared with 1 ml of ICG (2.5 mg/ml) added to 7 ml of distilled water. Ten microliters of fluid were added to a known volume of blank plasma, and the absorbance was determined.

Procedure for Direct Determination of PV and Derived Absolute BV
Before each dye injection, blood was withdrawn in heparinized syringes for hematocrit (Hct) determinations and blank plasma preparation. ICG (25 mg) was dissolved in 10 ml of sterile aqueous solvent, to produce an ICG solution of 2.5 mg/ml. Ten milligrams of dye were then injected, as rapidly as possible, into a venous port beyond the bubble trap (7). All syringes were weighed on a precision scale before and after injection, to establish the precise quantity infused. A comparison of the injected ICG amounts determined by weighing and read from the syringe marks demonstrated that approximately 99 ± 1.1% of the cited amounts were injected. Exactly 3 min after the end of the injection, sampling from the arterial port, into heparinized syringes, at 1-min intervals for 10 min (eight samples) was initiated. Samples were centrifuged at 3000 rpm for 10 min. The blank plasma sample was used to determine the baseline background absorbance at 805 nm. The absorbance of ICG dye in the timed plasma samples was then compared with the baseline values recorded at the same wavelength (8). Only 500 μl of plasma from each centrifuged sample was required, because of the use of half-microcuvettes.

Other Techniques
Patients were continuously monitored with respect to BP (BPS08 oscillometric system; Fresenius), relative BV changes (ultrasonic BV monitor; Fresenius) (9), and extracellular volume estimates (Hydra multifrequency, whole-body, bioimpedance system; Xitron Technologies, San Diego, CA). Hct values were measured with the Coulter counter method (STKS hematology flow cytometer; Beckman...

Table 1. Biometric data for patients studied

<table>
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<th>Variable</th>
<th>Mean</th>
<th>SD</th>
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</thead>
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<td>Age (yr)</td>
<td>61.1</td>
<td>10.1</td>
<td>50 to 78</td>
</tr>
<tr>
<td>Dry body weight (kg)</td>
<td>77.5</td>
<td>19.0</td>
<td>53.3 to 112.3</td>
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<td>BSA (m²)</td>
<td>1.91</td>
<td>0.27</td>
<td>1.54 to 2.42</td>
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<tr>
<td>Height (cm)</td>
<td>168.8</td>
<td>8</td>
<td>158 to 184</td>
</tr>
<tr>
<td>IDWG (liters)</td>
<td>2.3</td>
<td>1.01</td>
<td>0.8 to 4.15</td>
</tr>
</tbody>
</table>

*BSA, body surface area; IDWG, interdialytic weight gain.*

Figure 1. Relative blood volume (BV) profile during intermittent ultrafiltration for a patient in phase III. Arrows indicate the times of the four direct BV measurements using the dye.
Coulter, Palo Alto, CA). During these studies, UF volume was verified with collection of the ultrafiltrate in a measuring cylinder.

**Analyses**

The natural logarithm of the measured ICG dye concentration was plotted versus time for each PV measurement, and the best-fit linear regression for the data points was obtained (Figure 2). This allowed extrapolation of the straight line backward, to establish the logarithm of the initial dye concentration (offset from the regression line). The antilogarithm of the offset yielded the initial dye concentration in plasma at the time of injection. PV was calculated according to eq. 1 (Appendix).

BV was derived by using the Hct adjusted by a factor of 0.86, to correct for (1) the difference between Hct levels in the systemic circulation (Hctsys) and whole-body Hct levels (Hctbody) (F cell ratio, 0.90) and (2) trapped plasma (approximately 4%) (Hctbody = 0.90 × 0.96 × Hctsys = 0.86 Hctsys) (eq. 2, Appendix). PV measured during dialysis was compared with predialysis measurements after correction of the former for the volume of the extracorporeal circuit (internal fiber volume measured before dialysis with a Renatron analyzer and circuit volume measured with saline solution). PV readings recorded during the isovolemic phase were corrected for changes in plasma protein concentrations observed on the relative BV monitor (mean variation, 1.2%), to correct for any PV shifts induced by osmolar variations (8). The statistical methods used included Bland-Altman analyses (10) for comparisons of methods, t tests for comparisons of means (P < 0.05), linear regression analyses, and Pearson correlation tests. Statistical analyses were performed with the software package SigmaPlot (version 2.01; Sigma Chemical Co., St. Louis, MO) and curve-fitting software (Table Curve 2D).

**Results**

**Calibration Results**

There were marked differences between the absorbance slopes obtained with the two- and five-point calibration procedures (Figure 3). The two-point calibration seemed to consistently overestimate the slope for the three patients studied. The difference is possibly attributable to the assumed zero readings in the two-point calibration. The five-point calibration technique was deemed to be more precise and was used for the phase II and phase III studies. Four five-point calibration curves were obtained for all 19 studies performed in phases II and III (76 calibrations). The slopes obtained were highly consistent, with no significant intrindividual variation (Table 2). However, the background absorbance (intercept on the absorbance axis) varied considerably (Table 2).

**Phase I Results**

The use of a 5-mg bolus of ICG resulted in very low concentrations of ICG in the plasma, particularly in the tail of the dilution curve. Lack of precision in this area led to consistent overestimation of absolute PV. Administration of at least 10 mg of ICG (2.5 mg/ml), as used for most previous studies (7), was necessary to avoid this potential source of error. A site of injection close to the venous needle site was required.

**Table 2. Results of four five-point calibration curves for all 19 subjects in phases II and III**

<table>
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<tr>
<th>Calibration</th>
<th>Slope Mean ± SD</th>
<th>Intercept Mean ± SD</th>
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</thead>
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<tr>
<td>1</td>
<td>0.296 ± 0.013</td>
<td>0.047 ± 0.051</td>
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<td>2</td>
<td>0.296 ± 0.011</td>
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<td>3</td>
<td>0.285 ± 0.017</td>
<td>0.030 ± 0.031</td>
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<tr>
<td>4</td>
<td>0.285 ± 0.019</td>
<td>0.045 ± 0.045</td>
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</table>

* No significant differences between the measured calibration slopes were obtained (P > 0.05).
because injection into the bubble trap induced a delay in the decay curve. The problem was circumvented with the use of a venous sample port immediately adjacent to the venous needle site. Peak spectral responses were at 805 nm for plasma, blood, and hemolyzed blood (as determined with repeated laboratory wavelength scans). Spectral stabilization was sufficiently rapid for measurement of PV and BV and was very reproducible. The clarity of the plasma extracted from the blood samples was crucial. Significant interference was observed with hyperlipidemic, immediately postprandial, and grossly hemolyzed samples in the pilot phase. To determine the most appropriate sampling interval, the SD of measurements with different sampling times was calculated from the regression mean. This analysis confirmed that sampling beginning 3 or 4 min after ICG infusion led to the most consistent estimations. One subject, an elderly patient with congestive heart failure and atrial fibrillation, did not demonstrate complete mixing after 6 min. Samples were stable for approximately 8 h when stored at 4°C. The use of heparin did not affect the absorption spectra. No absorbance was detected at 805 nm in the dialysate aliquots obtained immediately after dye infusion. This confirmed that the dye was suitable for use during hemodialysis.

**Predialysis Values Compared with Isovolemic Dialysis Values**

Mean PV measurements during isovolemic dialysis compared well with measurements just before dialysis, with an acceptable mean difference of only 149 ± 341 ml between the predialysis PV and the first PV measurement during isovolemic dialysis (Figure 4).

**Reproducibility during Isovolemic Dialysis (Phase II)**

Three PV measurements during the first 1 h of isovolemic dialysis were consistent and highly reproducible. Correlation coefficients for the first and second measurements ($r^2 = 0.98$) were statistically significant (Figure 5). As a measure of repeatability, the mean ± SD for the differences between the first and second measurements were calculated as 33 ± 128 ml. The method mean SD was 356 ml, and the mean coefficient of variation of 4.07%. There were no traces of ICG in the baseline blank plasma samples, in repeated measurements. The mean half-life of the dye was 4.53 ± 1.5 min.

**Measurements during UF (Phase III)**

A significant reduction in PV was detectable during UF for all subjects except patient 2, who could not tolerate the fourth UF bolus and required saline infusion. The method was sensitive enough to detect this (Table 3). The mean arterial pressure was significantly correlated with directly measured circulating PV ($r = 0.70, P < 0.01$). There were no adverse reactions to the dye.

**Comparisons with Prediction Formulae**

Measured PV during the initial isovolemic period in both phase II and phase III were significantly correlated with extracellular fluid volumes, as measured by bioimpedance, for 14 patients ($r^2 = 0.73$) (Figure 6). Reliable estimates of extracellular fluid could not be obtained for five patients in phases II and III. The directly measured PV was also significantly correlated with body surface area (eq. 3, Appendix) (Figure 7).
The BV predicted with weight and height formulae, however, significantly (P < 0.0001) underestimated the absolute BV values measured with ICG. Regression lines for calculations with the Guyton, Hidalgo, Allen, and Baker methods (eqs. 4, 5, 6, and 7, Appendix) demonstrated a wide scatter, with means ± SD of the differences of 0.96 ± 1.2, −1.6 ± 1.7, −1.2 ± 1.4, and −1.4 ± 1.7 liters, respectively.

Discussion

Tracer methods for determination of PV and BV have a number of drawbacks, including the need for special equipment and often the requirement for the use of radioactive substances. The ICG method proposed by Bradley and Barr (4) did not initially gain widespread acceptance, probably because of their use of a nonstandard device designed for cardiac output measurements. The spectrophotometric method was first described by Schad et al. (11) for dogs. The method required central venous injection and sampling. The presence of an extracorporeal circuit and high-flow fistulae for hemodialysis patients greatly facilitates the use of this method for serial BV determinations during hemodialysis, eliminating the problem of central venous sampling. The favorable qualities of ICG are well documented, and spectrophotometric equipment is widely available. The ICG distribution space is similar to the PV. Estimates of PV obtained with this method have been demonstrated to be well correlated with estimates obtained with isotopic and Evans blue methods (7). Very few adverse events have been reported (12). The half-life of the dye in this study corresponded well to literature data (13).

There are some methodologic limitations of this technique. Because the half-life is short, compared with that of other tracers, accurate timing of the samples is critical. However, the short half-life makes ICG suitable for repeated measurements at short intervals, without the disadvantage of dye accumulation, as demonstrated in this study. General disadvantages include the need for a calibration curve for each patient and intraindividually varying conditions during hemodialysis. We have demonstrated that, with precise five-point calibration, consistent slopes can be obtained. The intercepts for the blank plasma samples were significantly different, presumably because of varying plasma compositions during dialysis. This finding suggests that a single five-point calibration for each patient is sufficient, provided that the baseline absorbance (intercept on the absorbance axis) is determined before each measurement. The slight variations between predialysis and isovolemic dialysis readings in phase II could be attributable to volume shifts induced by osmolar variations or dead-space volumes in the fistula needle before dialysis. Platelet counts and serum albumin concentrations may affect the disappearance rate. The maximal removal rate depends largely

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<th>PV3 (ml)</th>
<th>PV4 (ml)</th>
<th>BV1 (ml)</th>
<th>BV2 (ml)</th>
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a BV, blood volume; PV, plasma volume; pre – post weight, net weight loss during dialysis.

Figure 6. Regression line for PV (PV_{ICG}) versus extracellular fluid volume (ECF_{bioimpedance}) during the initial isovolemic dialysis period (r^2 = 0.73, P < 0.001).
on lecithin cholesterol acyltransferase and cholinesterase (14), which may introduce errors in hyperlipidemic samples. Dissociation between the maximal removal rate and the disappearance rate is possible with liver diseases such as cirrhosis and obstructive jaundice.

Except for these minor drawbacks, the method is highly reproducible, with a variation (coefficient of variation, 4.07%) well within the limits of other tracer methods (e.g., 6.5% for the radioimmunolabeled HSA method and 17% for the Evans blue method) (15). The variations are likely to be attributable to variations in blood-sampling techniques, variations in the stability of the physiologic parameters for the subjects, errors in Hct measurements, errors attributable to trapped plasma (3 to 4%), and unmeasured stromal proteins (which can cause underestimations of up to 1%, or 0.86 ml/liter blood). In agreement with other studies, we observed complete dye mixing after 3 min for all subjects in phases II and III (16). Analysis of plots of the logarithmic data facilitates identification of the moment of complete mixing. Mixing times may vary, especially among subjects with circulatory failure.

Indirect estimates of BV based on anthropometric data significantly underestimate directly measured BV changes, especially at the upper end of the range, possibly because the estimates are derived from databases and meta-analyses for normal healthy populations (17). However, we have demonstrated that, within the hemodialysis population, directly measured volumes exhibit good correlations with extracellular fluid volumes (Figure 6) and body surface areas (Figure 7). The ICG-derived absolute BV has hemodynamic significance and might play a central role in preserving vascular stability during UF.

In conclusion, ICG is a suitable tracer for repeated PV determinations among subjects undergoing hemodialysis. This method provides excellent reproducibility, can be performed in most laboratories, and provides a reference method for further research on hemodynamic instability during dialysis.

Acknowledgments
This study was supported by grants from Fresenius Medical Care (Germany). We thank D. Murray and S. Atkins for technical assistance.

Appendix
The following formulae have been used for calculations of PV, BV (18,19), and body surface area (20).

\[
PV = \frac{\text{dye infused (mg)}}{\text{plasma dye concentration (mg/liter)}}
\]

\[
BV = \frac{\text{plasma volume (dye)}}{1 - (\text{Hct}_{\text{sys}})}
\]

Body surface area =

\[
71.84 \times \text{body weight}^{0.425} \times \text{height}^{0.725}
\]

Formulae for deriving BV have been reported by Guyton et al. (21),

\[
BV = \frac{5}{70} \times \text{body weight}
\]

by Hidalgo et al. (22),

\[
BV = 0.367 \times \text{height}^3 + 0.0322 \times \text{weight} + 0.60
\]

by Hidalgo et al. (22),

\[
BV = 0.0417 \times \text{(0.414 for female subjects) \times height}^3 + 0.0450 \times \text{(0.0328 for female subjects) \times weight} - 0.03
\]

and by Baker et al. (22),

\[
BV = 0.0193 \times \text{height}^{0.725} \times \text{weight}^{0.425}
\]

References

Figure 7. Relationship of PV measured with ICG and body surface area. The best-fit linear regression is represented by \( y = 4505.5x - 4217.7 \) (\( r^2 = 0.82, P < 0.001 \)).