

Measurement of Cardiac Output and Blood Volume During Hemodialysis with Fluorescent Dye Dilution Technique

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Abstract—Intradialytic hypotensive events (IDH) accompanied by deleterious decreases of the cardiac output complicate up to 25% of hemodialysis treatments. Monitoring options available to track hemodynamic changes during hemodialysis have been found ineffective to anticipate the occurrence of IDH. We have assembled opto-electronic instrumentation that uses the fluorescence of a small bolus of indocyanine green dye injected in the hemodialysis circuit to estimate cardiac output and blood volume based on indicator dilution principles in patients receiving hemodialysis. The instrument and technique were tested in 24 adult end-stage renal failure subjects during 64 hemodialysis sessions. A single calibration factor could be used across subjects and across time. Intra-subject variability of the measurements over time was <10%. Stroke volume index (SVI) (mean \pm SEM = 34 ± 1 vs. 39 ± 2 mL m⁻²) and central blood volume (CBV) index (783 ± 36 vs. 881 ± 33 mL m⁻²) were lower at the beginning of the sessions in which IDH eventually occurred. Cardiac index, SVI, and CBV index decreased with hemodialysis in all treatment sessions but the decrease was more intense in the IDH sessions. We conclude that hemodynamic monitoring can be implemented in patients receiving hemodialysis with minimal disruption of the treatment and could help understand intradialytic hypotension.

Keywords—Fluorescence, Indocyanine green, Hemodynamic monitoring, Cardiac output, Blood volume, Hemodialysis, Intradialytic hypotension, Near-infrared fluorescence spectroscopy.

INTRODUCTION

The cardiovascular response to hemodialysis and ultrafiltration (HD/UF) is complex⁷ and the cardiovascular system's ability to adapt to a reduction of the plasma volume is a determining factor in the occurrence of intradialytic hypotensive events (IDH).⁴ Such events complicate up to 25% of HD/UF treatments.²⁵ Hypotensive events cause significant discomfort, and their repeated occurrence has been implicated in the end-organ damage that accompanies end-stage renal disease.^{14,30} Cardiac output (CO) decreases gradually during HD/UF^{1,3} and the trend is exaggerated when blood pressure drops.^{26,27} Blood pressure monitoring alone is ineffective in anticipating IDH in a subpopulation of unstable patients in whom CO drops substantially.⁴ These considerations support the development of a practical method for monitoring CO during HD/UF.

Various approaches based on indicator dilution methods,^{9,24,28,29,32} thoracic electrical impedance monitoring,^{12,33} or continuous non-invasive blood pressure measurements³ have been proposed. These approaches have remained experimental, in part because they have been difficult to implement in the clinical environment. We previously described an indicator-dilution method for assessing CO^{21–23} that relies on measuring the fluorescence of injected indocyanine green (ICG), a non-diffusible inert dye routinely used for hemodynamic measurements. Implemented with a superficial skin probe, the method was validated against the thermodilution method with an average error of 10% for single-point CO mea-

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measurements.²³ In the HD/UF environment, the measurement probe can be applied over the translucent arterial catheter without modifying the hemodialysis circuitry.

With the fluorescent dye dilution technique, CO can be measured every few minutes to obtain a comprehensive picture of the cardiovascular response during HD/UF. The dilution curves can be analyzed to estimate the central blood volume (CBV), a validated measure of cardiac preload.^{4,22} Blood volume (BV) can be estimated as the volume of blood in which injected ICG mixes homogeneously after multiple passes in the circulation and after correction for metabolic disposal of the dye.²³ Hypovolemia plays an important role in the development of IDH, and measurements of CBV and BV could help anticipate IDH events.^{14,29} BV measured in this way is an absolute estimate of the circulating BV in contrast to the relative BV which represents a change from an unknown baseline, and which has not been predictive of IDH events.^{6,14,27}

This study had two main goals: (1) to demonstrate that an instrument prototype based on the fluorescent dye dilution technique could be used to measure reliably and simply CO and BV repeatedly during HD/UF sessions without interference with the patient's hemodialysis treatment; (2) to examine if the fluorescent dye dilution technique can help identify differences in the patterns of variation of the CO and BV between HD/UF sessions complicated by IDH and those free of IDH.

MATERIALS AND METHODS

Instrumentation

The ICG fluorescence measurement system was similar to those used in our previous studies.^{20,22} A 785 nm near-infrared laser diode (Fermion 1, Micro Laser Systems, Garden Grove, CA) was connected to a custom-designed fiber optic probe (Leoni Fiber Optics, Williamsburg, VA) to excite the fluorescence of ICG in arterial blood circulating from the subject toward the hemodialysis machine (Fig. 1). The 2-m long probe fibers comprised one 100 μm illumination fiber surrounded by a bundle of twenty-three 100 μm fluorescence detection fibers arranged in an annulus (0.35–0.50 mm radius) around the illumination fiber. The placement of the detection fibers was selected based on Monte-Carlo simulations of ICG fluorescence detection in a blood medium which showed that the retro-reflected fluorescence intensity was most intense and least sensitive to variations of the hematocrit (Hct) when captured near the illumination entry point.²⁰ The probe tip was held perpendicular and in close contact

with the blood tube within a custom-designed metallic cylindrical holder which comprised a groove in which the blood tube was inserted and held firmly in place. The probe holder was positioned between the subject's arterial cannula and the entry point of the hemodialysis machine, 20–30 cm away from the cannula. The retro-reflected fluorescence signal was channeled by the fiber probe to a photomultiplier module (H5784-20, Hamamatsu, Bridgewater, NJ). An 830 nm narrow band-pass interferential filter (Melles Griot, Carlsbad, CA) in front of the photomultiplier window prevented most of the illumination light reflected by the blood tube from reaching the detector. The 785 nm illumination and 830 nm measurement wavelengths were selected to maximize the intensity of the ICG fluorescence emission.²¹ A lock-in amplifier (SR 810, Stanford Research Systems, Sunnyvale, CA) modulated the laser light at 1 kHz (average output power = 4 mW) and amplified the synchronous photomultiplier output. The lock-in amplifier output signal was low-pass filtered (-3 dB critical frequency = 16 Hz), visualized, and recorded at 40 samples/s with an electronic

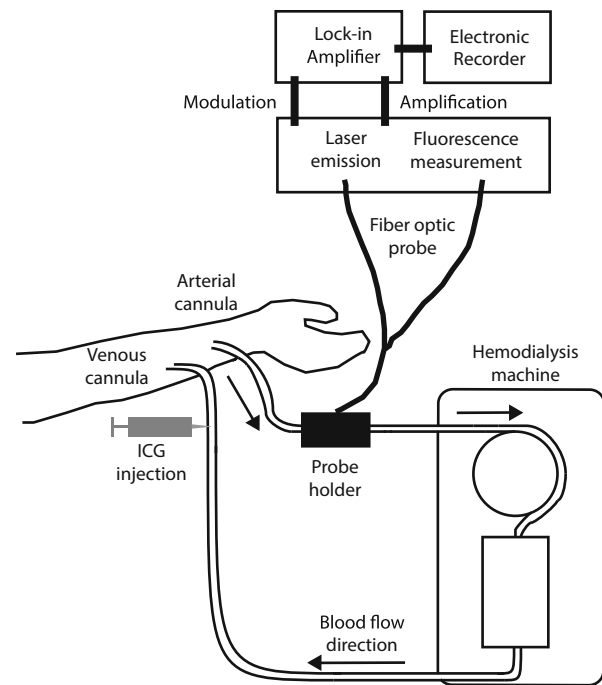


FIGURE 1. Experimental setup for measurement of hemodynamic parameters with ICG fluorescent dye dilution technique during hemodialysis. The distal end of the fiber optic probe was held in snug and stable contact with the hemodialysis tube by a black metallic probe holder. The probe channeled the laser emission at 785 nm to the blood tube and the retro-reflected indocyanine green (ICG) fluorescence back to the photomultiplier detector. ICG was bolus injected into a venous port located 15–20 cm from the venous cannula and its fluorescence was measured at level of the probe holder 20–30 cm distally from the arterial cannula.

chart recorder (Powerlab, AD Instruments, Colorado Springs, CO).

Probe Calibration

The measurement system was calibrated to convert the ICG blood fluorescence intensity readings into circulating blood ICG concentration values.^{20,23} A 16.7 mg dose of ICG in 5 mL 5% dextrose solution was rapidly injected in the subject's venous cannula, and allowed to circulate and uniformly mix in the circulating BV which occurred in about 2–3 min. Five 5-mL blood samples were rapidly withdrawn from the arterial cannula in heparinized syringes every 2 min starting 3 min after the ICG injection while the corresponding fluorescence reading was marked on the electronic chart. The blood samples were preserved on ice and processed within the hour to measure the ICG concentration at the time of the fluorescence readings. The samples were spun at 3000 rpm for 5 min to separate the plasma. ICG plasma concentration was read

in a calibrated spectrophotometer (UV-Vis 1800, Shimadzu, Columbia, MD) for wavelengths between 750 and 900 nm. Blood Hct measured from one of the blood samples was used to convert the ICG concentration in plasma to ICG concentration in blood. In this way, the 5 measured fluorescence readings were regressed against the 5 corresponding values of the blood ICG concentration to establish the linear calibration relationship.

Subject Recruitment and Experimental Protocol

Twenty-four end-stage renal disease adult subjects who had been on maintenance hemodialysis three times a week for at least 3 months were recruited from a community outpatient dialysis center affiliated with the University Of Southern California Keck School Of Medicine. The subjects gave their written informed consent to the experimental protocol approved by the University of Southern California Institutional Review Board. Exclusion criteria included allergy to iodine or seafood, significant access recirculation within 1 month prior to the study, clinical diagnosis of congestive heart failure, elevated liver enzyme levels, and contagious blood disease. Subjects were preferentially recruited among patients whom the dialysis center staff had identified as having a tendency to experience intradialytic morbid events. The medical record was reviewed by the consenting physician during the informed consent. Biometric data for the subjects is provided in Tables 1 and 2.

The protocol accommodated with minimal disruption the hemodialysis treatment such that each subject was studied three times over a 3 to 4-week period. HD/UF was delivered in a standard fashion by the dialysis

TABLE 1. Subject characteristics (N = 24).

Female	10 (42%)
Ethnicity	H: 21; AA: 2; As: 1
Diabetes mellitus	12 (50%)
Beta blockers	12 (50%)
Age (years)	51.3 ± 14.1
Body weight (Kg)	70.8 ± 16.7
Height (cm)	165.4 ± 7.4
BMI	26.0 ± 6.4

Characteristics of the subjects enrolled in the study (mean ± SD). Ethnicity (H, Hispanic; AA, African-American; As, Asian), number of subjects with Diabetes Mellitus and using beta blocker medicines are indicated.

TABLE 2. Session characteristics (N = 64).

	IDH sessions	Non-IDH sessions	<i>p</i>
Number of sessions	20 (31%)	44 (69%)	0.03
Female	13 (65%)	11 (25%)	0.03
Age (years)	57.5 ± 10.9	49.0 ± 14.8	0.03
Body weight (Kg)	75.2 ± 18.7	71.0 ± 15.3	0.29
Height (cm)	162 ± 5	169 ± 7	0.001
BMI	28.8 ± 7.0	25.3 ± 6.2	0.05
Baseline hematocrit (%)	35.8 ± 2.9	36.7 ± 3.5	0.4
Years on dialysis	3.9 ± 3.4	4.6 ± 2.3	0.27
Ultrafiltration rate (mL h ⁻¹)	1135 ± 204	1001 ± 235	0.03
Specific ultrafiltration rate (mL h ⁻¹ Kg ⁻¹)	15.9 ± 4.4	14.5 ± 3.7	0.24
Total ultrafiltrated volume (mL)	3617 ± 698	3508 ± 864	0.58
Dialyzer blood flow (mL min ⁻¹)	400 ± 32	419 ± 24	0.02
Duration of dialysis (h)	3.42 ± 0.33	3.54 ± 0.31	0.18

Characteristics for the 64 HD/UF sessions retained for data analysis sorted based on whether intradialytic hypotensive (IDH) events were recorded in the session (mean ± SD). Each session is counted as one entry and the subject characteristics for that session are recorded and averaged, adjusting for repeated measurements within a subject. The IDH sessions were observed significantly more frequently in older subjects, females, and subjects with a higher BMI. Ultrafiltration rate was slightly higher in the IDH sessions. There were no differences in the specific ultrafiltration rate or the treatment duration between IDH and non-IDH sessions.

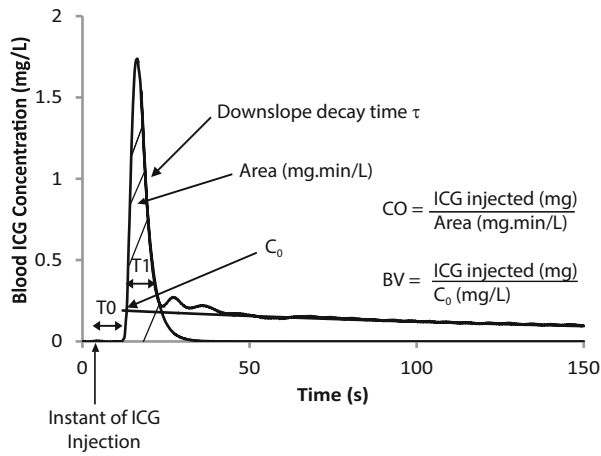


FIGURE 2. Analysis of the fluorescence recording after conversion into circulating blood indocyanine green (ICG) concentration. The area under the first pass trace (hashed) is estimated to compute the cardiac output (CO). The slow decay trend representing metabolic disposal of the ICG is back-extrapolated to estimate C_0 , the concentration that would be obtained if the mass of injected ICG was mixed in the total circulating blood volume (BV). Time T_0 represents the interval between the ICG injection and the appearance of ICG at the level of the detector while time T_1 is mean transit time of the first pass trace. The duration ($T_0 + T_1$) is used to estimate the central blood volume CBV.

center staff and was preceded by placement of an arterial and a venous cannula in the subject's access site. HD/UF equipment comprised the hemodialysis machine (Fresenius 2008 K, Fresenius Medical Care, Waltham, MA) set at a constant UF rate, a standard medical grade PVC translucent blood tubing set (6.5 mm OD, 4.5 mm ID) which bore the optical probe, and the dialyzer (Revaclear, Gambro Renal Products, Lakewood, CO). The subjects rested recumbent during the treatment with a blood pressure cuff on the arm not used for dialysis and behaved according to their routines. No adverse event was observed during or immediately after the studies.

ICG fluorescence traces were measured every 15 min approximately during the HD/UF treatment (8–15 times, median = 12). For each measurement, 1.4 mg ICG dissolved in 2 mL of 5% dextrose solution was rapidly injected in the port of the dialysis circuitry nearest to the venous cannula. Systolic and diastolic blood pressures (SBP and DBP), and heart rate (HR) were measured just prior to the ICG injection. The instant of injection, the ultrafiltrated volume (UV), and the subject's behavior were noted on the electronic chart recorder with the fluorescence intensity trace. Within 10 s of the injection, a typical indicator dilution trace was observed (Fig. 2). The fluorescence signal returned to baseline within 5 min. A 1 mL arterial blood sample was obtained after every other injection

to assess blood Hct changes with ultrafiltration. Calibration of the probe took place after the second ICG injection to capture the hemodynamic state of the subject at the beginning of the study as soon as the dialysis center staff initiated the HD/UF treatment.

Derivation of Calibration Relationship

The five fluorescence intensities $F(t_i)$ measured during the calibration procedure were scaled by the intensity reading obtained prior to the injection of ICG ($F(t_0)$):

$$F_{\text{scaled}}(t_i) = \frac{F(t_i) - F(t_0)}{F(t_0)} \quad (1)$$

Scaling by $F(t_0)$ accounted for experimental fluctuations of the illumination intensity, photomultiplier gain, and optical coupling at the probe-tube interface between studies. Blood ICG concentration at time t_i measured by spectrophotometry $C(t_i)$ was related to the scaled fluorescence $F_{\text{scaled}}(t_i)$ in linear relationship that appeared largely constant between studies. Therefore, the calibration data points measured in all studies were combined to determine a single calibration factor (slope of the regression line between F_{scaled} and C) that was used to analyze all the fluorescence curves.

Derivation of Hemodynamic Parameters

The ICG fluorescence intensity was scaled by the photomultiplier signal reading averaged over 20 s prior to the injection (Eq. 1) and converted to a blood ICG concentration measurement using the fluorescence calibration factor. Parameters CO and BV were estimated as previously described.^{21,23} The descending part of the first pass ICG concentration trace (Fig. 2) was approximated by a descending exponential to eliminate the recirculation. CO was computed by dividing the dose of injected ICG by the area under the first pass curve. Stroke volume (SV) was obtained by dividing CO by the HR at the time of the ICG injection.

The slow descending ICG concentration trace observed after mixing of the dye in the BV was approximated by a decreasing exponential function $C(t) = C_0 e^{-t/\theta}$. The exponential function was back extrapolated to the instant at which the ICG was first detected to compute the estimated concentration C_0 that would be measured by mixing the injected ICG in the circulating BV. Volume BV was computed by dividing the mass of injected ICG by the concentration C_0 and included the volume of blood in the hemodialysis circuitry (~200 mL).

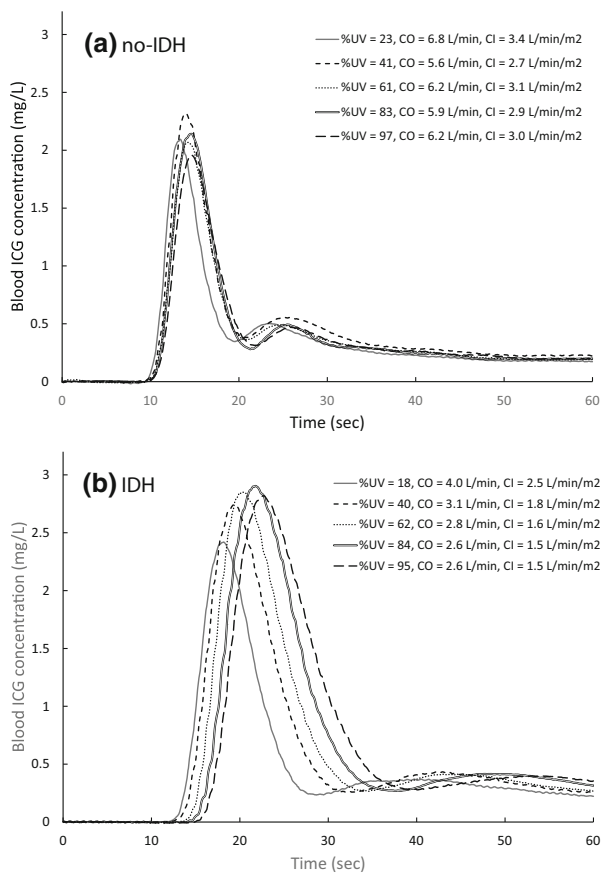


FIGURE 3. Indocyanine green (ICG) dilution curves recorded during the course of hemodialysis sessions without IDH event (3A) and with IDH event (3B). Progression through the session is represented by the %UV, the UV at the time of ICG injection expressed as percentage of the total UV in the session. Time 0 corresponds to the instant of ICG injection marked with an electronic switch on the chart recorder. For the session without IDH event (3A), total UV was 4000 mL. Cardiac output (CO) decreased initially from 6.8 L min⁻¹ (%UV = 23) then stabilized around 6.0 L min⁻¹ (%UV = 41–97). Duration (T₀ + T₁) which comprised the lag between the ICG injection and the appearance of the dye at the level of the probe (T₀) plus the mean transit time under the first pass curve (T₁) increased from 14.5 to 16 s. Central blood volume (CBV = CO * [T₀ + T₁]) slightly decreased from 1.7 to 1.5 L. The ICG curves changed more substantially in the session with IDH event (3B) in which the total UV was 3500 mL. A marked progressive increase of the area under the first pass curve corresponds to a decrease of CO from 4.0 L min⁻¹ (%UV = 18) to 2.6 L min⁻¹ (%UV = 84–97). Duration (T₀ + T₁) increased from 20 to 26 s and CBV decreased from 1.3 to 1.0 L.

The time between the instant of injection of the dye and the beginning of the dye curve (T₀) was estimated as was the mean transit time (T₁): $T_1 = \int (t - T_0) \cdot C(t) dt / \int C(t) dt$. CBV was computed as (T₀ + T₁) * CO.^{9,10,18} Parameters CO, SV, and CBV were normalized by body surface area calculated with the Mosteller formula to estimate the cardiac index (CI), stroke volume index (SVI), and

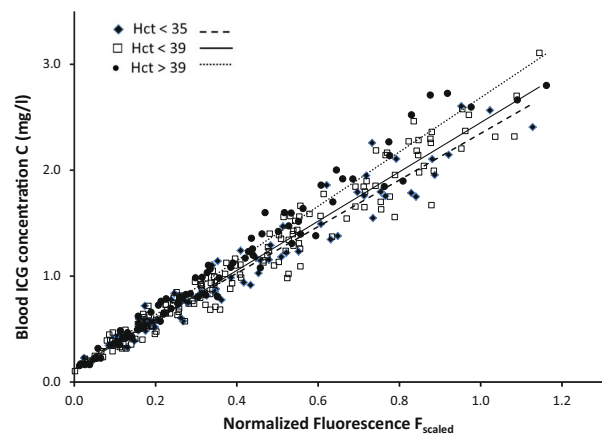


FIGURE 4. Calibration data points expressing blood ICG concentration as a function of the fluorescence intensity normalized by the detector reading obtained prior to the ICG injection. The data points measured in all experimental studies are sorted in three groups based on the hematocrit at the time of the calibration. Small but significant differences in the slope of the calibration lines were identified which are consistent with the fact that when the hematocrit is higher, illumination and fluorescent lights are absorbed more intensely by blood such that the remitted fluorescence intensity decreases.

central blood volume index (CBVI). BV was normalized by dry weight to compute the specific blood volume (BVI).

Statistical Analysis

Subject characteristics and initial values of the hemodynamic parameters were compared between HD/UF sessions with an IDH event and sessions without IDH event using repeated measures mixed effects modeling to account for repeated observations within subjects across sessions. An IDH event was defined as a decrease in systolic blood pressure SBP ≥ 20 mmHg or a decrease in mean arterial pressure ≥ 10 mmHg compared to the blood pressure measured at the beginning of the HD/UF session and associated with clinical symptoms and the need for staff intervention.^{8,13} In all mixed effects models, IDH was the fixed effect.

To account for repeated measures, longitudinal linear mixed effects modeling was used to describe trends in the hemodynamic parameters as a function of percent volume removed (%UV) in the IDH and non-IDH studies. The parameter %UV was found to be superior to the UV to model the longitudinal component since measures of the model fit improved when %UV was used. Data collection points were nested within a session, which was nested within subjects. Fixed effects included IDH, %UV, and the interaction IDH \times %UV. To account for different total volumes

TABLE 3. Measurements of hemodynamic parameters and hematocrit (mean \pm SEM) at the beginning of HD/UF for sessions with or without an intradialytic hypotensive (IDH) event.

	IDH sessions	Non-IDH sessions	<i>p</i>
N sessions	20	44	
UV (mL)	532 \pm 69	512 \pm 54	0.70
CO (L min ⁻¹)	4.70 \pm 0.27	4.93 \pm 0.19	0.50
CI (L min ⁻¹ m ⁻²)	2.60 \pm 0.12	2.74 \pm 0.11	0.41
SV (mL)	61.5 \pm 3.2	69.6 \pm 2.1	0.04
SVI (mL m ⁻²)	33.9 \pm 1.4	38.9 \pm 1.5	0.04
CBV (L)	1.40 \pm 0.07	1.58 \pm 0.05	0.03
CBVI (mL m ⁻²)	783 \pm 36	881 \pm 33	0.05
HR (beats min ⁻¹)	77 \pm 3	70 \pm 1	0.006
BV (L)	3.91 \pm 0.19	4.03 \pm 0.14	0.59
BVI (mL Kg ⁻¹)	52.5 \pm 2.7	59.5 \pm 2.8	0.13
SBP (mmHg)	143 \pm 6	147 \pm 4	0.55
DBP (mmHg)	76 \pm 3	80 \pm 3	0.35
Hct (%)	36 \pm 1	37 \pm 1	0.40

UV, volume ultrafiltrated at the time of the first measurement; CO, cardiac output; CI, cardiac index; SV, stroke volume; SVI, stroke volume index; CBV, central blood volume; CBVI, central blood volume index; HR, heart rate; BV, blood volume; BVI, specific blood volume; SBP, systolic blood pressure; DBP, diastolic blood pressure; Hct, hematocrit. Parameters SV, SVI, CBV, and CBVI were lower while HR was higher for the IDH sessions.

The *p* values refer to univariate generalized estimating equations to account for repeated sessions within participants.

removed across individuals and sessions, total volume removed was an *a priori* covariate.

A small effect of blood Hct on the relation between scaled fluorescence intensity F_{scaled} and blood ICG concentration C was anticipated.²⁰ To investigate this effect, the measurement pairs (F_{scaled} , C) were sorted in three groups based on Hct at the time of the calibration: Hct < 35, Hct < 39, and Hct > 39. The resulting linear regression slopes were compared with a general linear model in which concentration C was the dependent variable, F_{scaled} was the covariate, and the Hct group was a fixed effect. Except where noted, data are reported as mean \pm SEM. All analyses used SPSS (v.21).

RESULTS

Sixty-four HD/UF sessions were retained for analysis of the effect of IDH on hemodynamics (Tables 1 and 2) after we excluded 8 sessions in which the total volume removed was ≤ 2 L since such sessions are very rarely accompanied by IDH events. At least one IDH event was observed in 20 of the 64 retained sessions (31%).

Morphology of ICG Dilution Curves During HD/UF

ICG dilution curves became progressively taller and wider as HD/UF progressed (Fig. 3). The area under the first pass dilution curve increased which corresponded to a gradual decrease of the CO. This tendency was accentuated in HD/UF sessions

accompanied by IDH events. Time T_0 between the instant of injection in the venous cannula and the appearance of the fluorescent dye at the level of the detection probe gradually increased to reflect a decrease of the blood flow rate between the locations of ICG injection and fluorescence measurement. Mean transit time T_1 also increased. However, CO usually decreased more acutely than the duration ($T_0 + T_1$) increased, resulting in a progressive decrease of the CBV. A noticeable secondary rise of the ICG concentration after the first pass curve corresponded to the recirculation of the dye in front of the measurement probe.²¹ Thereafter, the ICG signal gradually decreased and returned to baseline in 5–10 min.

Reproducibility of Hemodynamic Measurements

Data from four studies in two subjects excluded from the main analysis because the total UV in each study (average 0.7 L) was well below the 2 L threshold was used to assess the intra-subject reproducibility of the measurements. Parameters CI, BVI, and CBVI were independent of %UV in these studies. In the first subject, a male with a body mass index = 27.6 and elevated but stable mean blood pressure = 127 mmHg, CI averaged 4.2 ± 0.3 L min⁻¹ m⁻² (mean \pm SD) over 3 studies (34 measurement points) with a coefficient of variation (CV = SD/mean) = 8%. The corresponding values for BVI and CBVI were 76 ± 10 mL Kg⁻¹ (CV = 13%), and 1370 ± 120 mL m⁻² (CV = 8%). There was no significant difference between the parameter values obtained in the 3 studies which took place over a 3-week period. The

TABLE 4. Mixed effect modeling of measured parameters using the presence or absence of intradialytic hypotensive event (IDH), the volume ultrafiltered expressed as a percentage of the total volume removed in a session (% UV), and their interaction as independent variables and the absolute total volume removed (Total UV) as a covariate.

	Intercept		IDH		% UV		IDH X % UV		Total UV	
	Estimate	p	Estimate	p	Estimate	p	Estimate	p	Estimate	p
CI (L min ⁻¹ m ⁻²)	2.77 ± 0.12	0.11	-0.18 ± 0.11	<0.001	(-9.5 ± 1.0) · 10 ⁻³	<0.001	(1.8 ± 1.4) · 10 ⁻³	0.20	(-3.6 ± 3.1) × 10 ⁻⁵	0.24
SVI (mL m ⁻²)	44 ± 1.5	<0.001	-5.9 ± 1.4	<0.001	-0.11 ± 0.01	<0.001	(1.2 ± 2.0) · 10 ⁻²	0.56	(-1.2 ± 0.2) × 10 ⁻⁴	0.003
CBVI (mL m ⁻²)	905 ± 37	0.001	-122 ± 35	<0.001	-1.6 ± 0.3	<0.001	0.15 ± 0.54	0.79	(-7.8 ± 9.7) × 10 ⁻³	0.42
HR (beats min ⁻¹)	65.6 ± 1.9	0.002	5.8 ± 1.9	0.002	0.02 ± 0.02	0.33	-0.01 ± 0.03	0.75	(1.4 ± 0.5) · 10 ⁻³	0.75
BVI (mL Kg ⁻¹)	60.7 ± 2.6	0.001	-8.0 ± 2.4	0.001	-0.06 ± 0.02	0.01	0.07 ± 0.03	0.03	(-5.3 ± 6.5) × 10 ⁻⁴	0.42
SBP (mmHg)	125 ± 5	0.80	1.1 ± 4.2	0.001	-0.14 ± 0.04	0.001	-0.40 ± 0.06	<0.001	(7.3 ± 1.2) × 10 ⁻³	<0.001
DBP (mmHg)	71 ± 3	0.002	-8.7 ± 2.8	0.002	-0.05 ± 0.03	0.05	-0.13 ± 0.04	<0.001	(3.5 ± 0.8) × 10 ⁻⁴	<0.001

Stroke volume index (SVI) and central blood volume index (CBVI) were lower in the IDH sessions and decreased significantly with %UV. Cardiac index (CI) decreased with %UV but the effect of IDH on CI was masked by the significantly higher heart rate (HR) consistently observed in the IDH sessions. Estimates are reported ± SEM.

second female subject had a smaller body mass index = 21.0 and a stable mean blood pressure = 98 mmHg. In that subject, CI, BVI, and CBVI averaged $3.1 \pm 0.2 \text{ L min}^{-1} \text{ m}^{-2}$ (CV = 6%), $50 \pm 4 \text{ mL Kg}^{-1}$ (CV = 9%), and $750 \pm 30 \text{ mL m}^{-2}$ (CV = 4%) over 9 data points in one study.

Effect of Hematocrit on Calibration Factor

A small but statistically significant difference was found for the slope of the calibration relationship between blood ICG concentration C and scaled ICG fluorescence F_{scaled} for different ranges of the Hct (Fig. 4). The slope of the regression line was 9% higher for Hct > 39 and 6% lower for Hct < 35 when compared to the overall slope found using all the pairs (F_{scaled}, C).

Initial Values of Hemodynamic Parameters

Hemodynamic parameters were first measured for an UV = 532 ± 69 mL in the IDH sessions and 512 ± 54 mL in the non-IDH sessions (Table 3). The initial values of CBV, CBVI, SV, and SVI were lower in the studies in which IDH was eventually detected, suggesting that the subjects began HD/UF with a lower preload, which was associated with a lower SV. A higher HR compensated for the lower SV such that CO and cardiac index CI were not different. The other parameters were not statistically different.

Time Course of Hemodynamic Parameters During HD/UF

Parameters CI, SVI, and CBVI gradually decreased as HD/UF proceeded in all studies (Fig. 5). However, the percent change was larger in the IDH sessions: 30 vs. 19% for CI, 32 vs. 21% for SVI, and 16 vs. 13% for CBVI. Both IDH and the percentage of the total volume removed % UV were significant factors in the mixed effect modeling of SVI and CBVI (Table 4), indicating that these two parameters decreased more intensely as HD/UF progressed in the IDH sessions. HR was higher in the IDH sessions, without significant change as HD/UF progressed. The combined effect of IDH on SVI and HR was such that cardiac index CI decreased with % UV but IDH was not a significant factor in the mixed effect modeling of CI.

Specific blood volume (Fig. 5; Table 4) was lower in the IDH sessions when compared to the non-IDH sessions by approximately 8 mL Kg⁻¹ at the start of HD/UF (Table 4). Volume BVI decreased significantly with HD/UF, as shown by the significant IDH and %UV terms in the mixed effect modeling. Across all sessions, BVI decreased by approximately

$0.06 \times 100 = 6 \text{ mL Kg}^{-1}$ at the completion of HD/UF (Table 4). Pressures SBP and DBP decreased more intensely (Fig. 5) during the course of HD/UF in the

IDH sessions in comparison to the non-IDH sessions: 27 vs. 5% for SBP and 20 vs. 5% for DBP. The effects of IDH and % UV were manifest in the IDH, % UV,

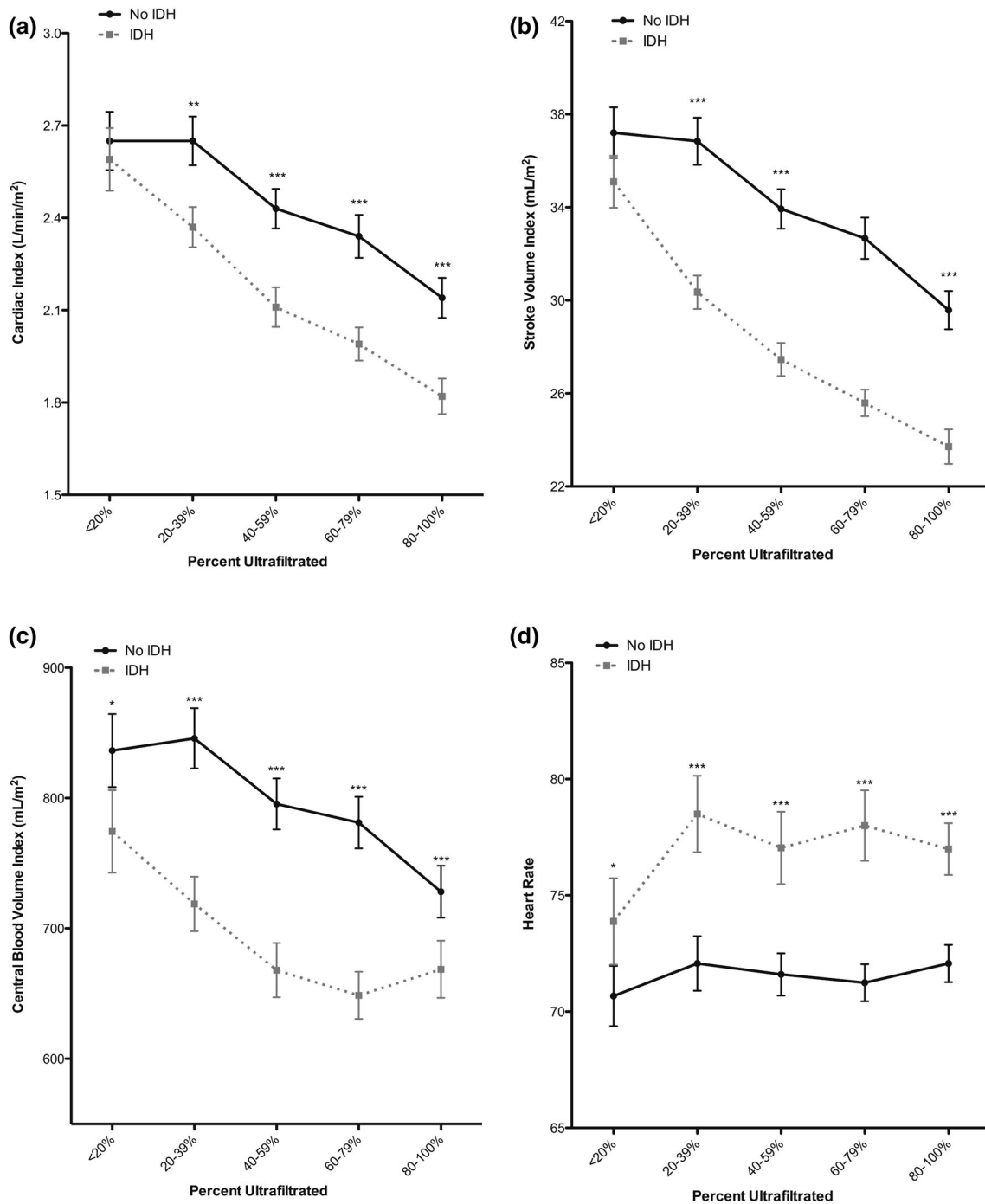


FIGURE 5. Variations of hemodynamic parameters ((a) CI; (b) SVI; (c) CBVI; (d) HR; (e) BVI, (f) SBP; (g) DBP) in sessions with IDH events and sessions without IDH events. CI, cardiac index; SVI, stroke volume index; CBVI, central blood volume index; HR, heart rate; BVI, specific blood volume; SBP, systolic blood pressure; DBP, diastolic blood pressure. All parameters are plotted as a function of %UV, the volume ultrafiltrated at the time of the measurement expressed as a percent of the total volume ultrafiltrated in the session. Each data point represents the average of all measurements within an interval of width equal to 20% of the total volume ultrafiltrated. Error bars represent the standard error of the mean (SEM). * <0.05 ; ** <0.01 ; *** <0.001 . The p -values are not adjusted for multiple comparisons.

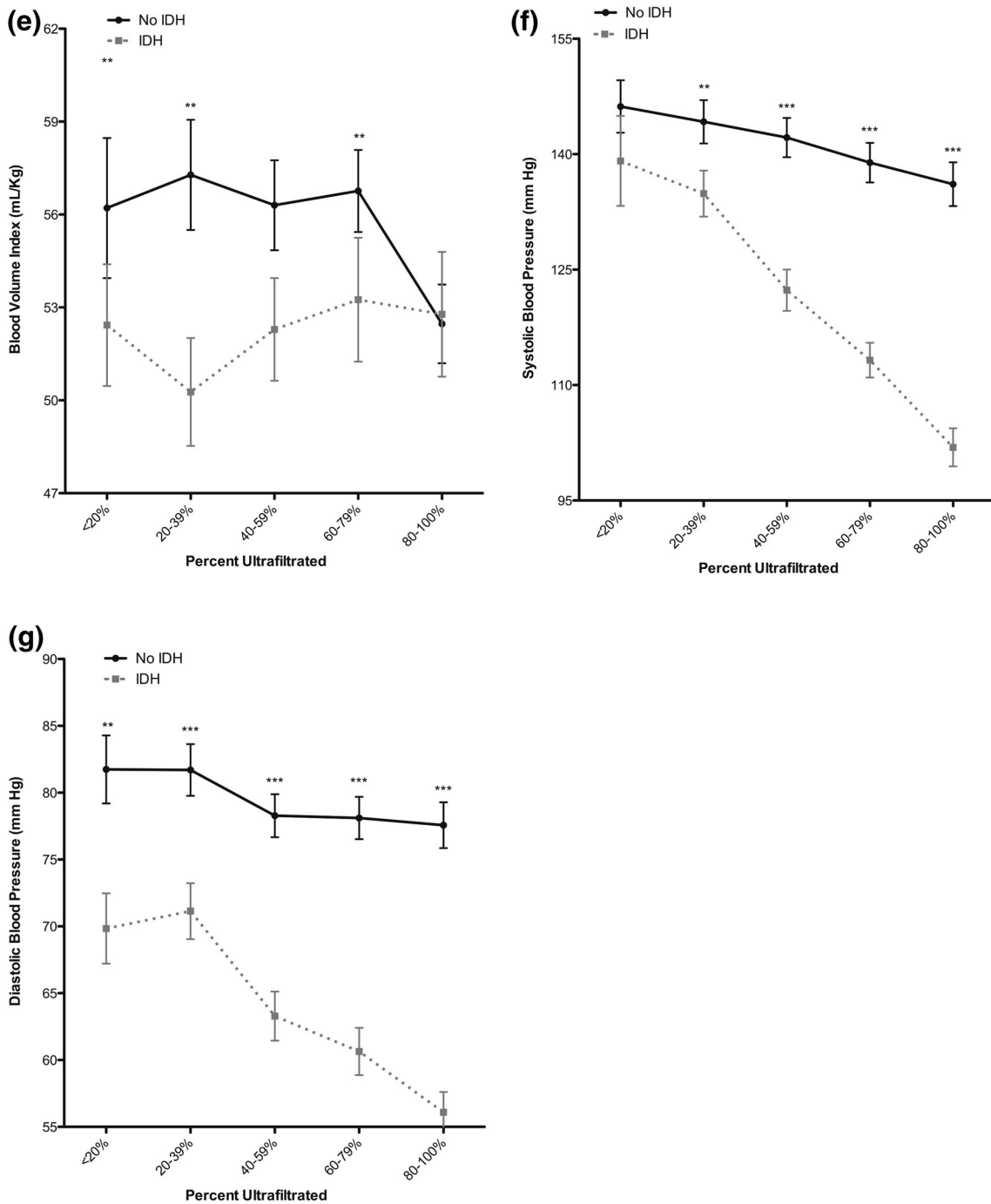


FIGURE 5. continued

and interaction terms of the mixed effect modeling of SBP and DBP (Table 4).

Blood Hct increased by approximately 3% between the beginning and the end of the HD/UF sessions, independently of whether IDH occurred in the session or not.

DISCUSSION

Our main findings were as follows. First, hemodynamic parameters, including CO, CBV, and BV can be estimated non-invasively with the fluorescent dye dilution technique during hemodialysis with simple

instrumentation and minimal changes to the patient's conventional treatment. Second, HD/UF sessions complicated by IDH exhibited lower initial CBV (a measure of cardiac preload), lower SV, and higher HR. Cardiac preload and SV changed more intensely as HD/UF proceeded in IDH sessions when compared to non-IDH sessions.

Use of ICG optical absorption to measure hemodynamic function during HD/UF has been described.^{25,29,32} We previously validated the measurement of CO and BV with the fluorescent dye dilution technique using a fiber optic probe placed on the skin surface.^{21–23} The probe was calibrated for each position on the skin to account for local variations of the vasculature and blood flow pattern in the tissue volume interrogated by the probe. The present study showed that a fluorescence probe fitting in a stable and duplicable contact around the transparent hemodialysis tubing can reliably measure circulating blood ICG concentration without having to repeat the calibration procedure for each study. The probe must be placed close to the arterial cannula to preserve the ICG dilution traces and avoid the distortion caused by circulation through the dialysis machine. The reproducibility of the hemodynamic measurements (coefficient of variation <10%) suggests that the technique can be used to track hemodynamic changes in patients receiving hemodialysis over extended periods of time.

The linear calibration relationship between fluorescence intensity and blood ICG concentration was stable across subjects and across studies in the same subject. A small dependence on blood Hct was identified, with a larger slope for the higher range of Hct. This result was consistent with the analysis of the relationship between fluorescence and blood ICG concentration obtained from Monte-Carlo simulations and empirical *in vitro* tests. As Hct increased, absorption of the illumination and fluorescent light in the blood decreased the fluorescence signal retro-reflected by the blood medium.²⁰ Using a single calibration factor as was done in our analysis slightly underestimated the blood ICG concentrations for the higher Hcts resulting in overestimations of CO, BV, and CBV. As Hct increased on average by 3% during the HD/UF sessions, these hemodynamic parameters likely decreased a little more toward the end of the sessions than we estimated by using a unique calibration factor. Technology for non-invasive measurement of the Hct during HD/UF could be used to account for Hct changes and improve the accuracy of the cardiovascular measurements.¹⁷

Measuring the fluorescence of ICG as opposed to its absorption was advantageous because the increased sensitivity allowed for substantially smaller ICG doses:

1.4 mg per hemodynamic measurement in our study vs. 10–20 mg in earlier studies.^{11,29} The fluorescent dye dilution curves presented characteristic shapes with little experimental noise and could be processed to extract CO and BV. The dye completely cleared from the blood in less than 10 min (half-life < 3 min), allowing for a comprehensive description of cardiac hemodynamics during the HD/UF treatment. Analysis of the ICG curves yielded the CBV, which can help interpret changes in cardiac function during HD/UF in terms of preload changes.^{5,19,32}

In our study, CBV was lower at the beginning of HD/UF in the IDH sessions, which resulted in a lower SV compensated by an elevation of the HR to maintain CO. As HD/UF progressed, CBV decreased more intensely in the IDH sessions, which coincided with larger CO and SV decreases. These results confirmed those of multiple studies that have shown marked decreases of CI and SVI during HD/UF,^{1,3,6,27,34} particularly in patients who experienced a decrease in blood pressure without IDH¹ or with IDH.¹² In one study, CI and SVI were lower at the start of HD in subjects who subsequently experienced IDH.¹² In other studies, CI and SVI were initially comparable in all subjects but diminished more markedly in those prone to IDH³⁴ or in those who developed IDH.²⁷ CBV decreased during HD/UF in non-IDH patients, particularly when the volume removed was > 3 L,^{31,32} as was the case in our subjects. The magnitude of the CBV decrease was correlated to the change in CI, SVI, and SBP.³²

Specific blood volume measured with our technique was comparable to the measurements obtained in hemodialysis patients with an optical absorption approach also based on ICG dilution,²⁹ but lower than the measurements obtained with other techniques.^{14,15} In part, the ICG dilution method probes the vascular space in which the dye readily disperses, particularly when a small dose of ICG is used. Hypo-perfused vascular areas which are not rapidly reached by the dye are only partially accounted for. Parameter BVI decreased slightly with HD/UF across all sessions. This observation was consistent with reports that relative BV changes are small and do not correlate with intradialytic blood pressure variations.^{2,6,14,27} Our results suggest that the BV in the central compartment represented by CBV was more informative to identify HD/UF sessions complicated by IDH.^{2,14,15}

Among the limitations of the study, the intensity of the ICG fluorescence remitted toward the measurement probe could be altered by changes in red cell volume consecutive to blood osmolarity changes and affect the estimated parameters.¹⁶ The fluorescent dye dilution technique was not validated against gold standard techniques such as the thermodilution method due to their invasiveness. The IDH-associated

pattern of decreased preload may not generalize to a community sample of a different demographic or apply to a population with greater disease acuity. The results could have been slightly biased by the fact that only 7 subjects had both IDH and non-IDH sessions, while 12 subjects did not experience IDH at all. Follow-up studies should examine the ability of the parameters SVI, CBVI used in combination with traditional measures, SBP, HR, and patient clinical history to predict the risk of occurrence of IDH.

In conclusion, we demonstrated that the fluorescent dye dilution technique could be used to reliably measure CO and BV in end-stage renal disease subjects receiving hemodialysis with little interference to their treatment. The technique identified differences in the variation patterns of the CO and CBV in hemodialysis sessions complicated by IDH. The proposed technology could pave the way for developing on-line monitoring systems that anticipate the risk of occurrence of IDH during hemodialysis.

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