Cardiovascular Monitoring by Pulse Dye Densitometry or Arterial Indocyanine Green Dilution

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Introduction

Noninvasive cardiac output (CO) monitoring has gained increasing clinical attention in recent years. Various methods have been developed, based on different techniques, with variation in reliability and clinical applicability. Pulse Dye Densitometry (PDD) uses the indicator dilution technique and requires only intravenous access. The indicator Indocyanine Green (ICG) is confined to the intravascular space due to its hydrophilic character and binding to plasma proteins and is cleared from the blood through the liver. ICG has been used frequently to determine cardiovascular parameters such as cardiac output, cardiac index, blood volume, liver blood flow, and lung-uptake of drug components like lidocaine through dye dilution. With the introduction of PDD, an adaptation of pulse spectrophotometry, it became possible to measure ICG noninvasively by means of transcutaneous spectrophotometry using a finger or nose probe.

Three validation studies of the measurement of the arterial blood ICG concentration versus pulse dye densitometry have been published so far. In these studies, blood samples were taken after recirculation had taken place. However, the phase of initial mixing, before recirculation of the dye, is the period in which the data are gathered for the estimation of cardiac output and central blood volume (CBV), since the area under the first pass curve is needed for the calculation of these parameters. In the study published by Sakka and colleagues, intravascular measurement of ICG using a fiberoptic device showed good agreement with transcutaneous measurement of ICG for the determination of total blood volume (TBV), moderately for CBV and could not be performed for CO, due to inaccurate detection of the first-pass curve. Consequently, validation of transcutaneous measurement of ICG by PDD versus ICG concentration measurements in arterial blood during the initial mixing phase is not available.

To evaluate the noninvasive measurement of ICG using pulse dye densitometry, we compared in a group of patients the ICG data measured using the PDD finger or nose probe to the ICG concentrations in arterial blood, and compared the cardiovascular parameters derived by these 2 methodologies.
Methods

Study design and subjects

The ICG concentrations determined noninvasively by PDD and invasively in arterial blood were taken from patients who participated in either of two yet unpublished pharmacological studies. In 10 patients ICG concentrations were measured using the PDD finger probe, in 10 other patients the nose probe was used. The studies were performed after obtaining approval from the Medical Ethics Committee of the Leiden University Medical Centre and the patients’ written informed consent. The patients were ASA physical status I or II and participated in the study prior to elective surgery. Exclusion criteria included a medical history of severe cardiovascular, respiratory, renal, hepatic, neurological or psychiatric disease, use of anti-hypertensive or anti-arrhythmic medication, pregnancy or lactation, and a history of hypersensitivity to amide local anesthetics or ICG.

Prior to the measurements a large venous cannula was inserted in the fossa cubiti and the radial artery was cannulated for gathering blood samples with a 22 G cannula. Placement of the finger probe was on the index finger and the nose probe on the right nasal wing. Cooling of the extremities was prevented to maintain signal quality. An experimental session only started when the PDD indicated a sufficient signal quality as measured by the finger or nose probe. Sufficient was judged a minimum of two out of five units as indicated on the DDG-2001. Each session started with the intravenous administration of 10 mg ICG (Infracyanine®), followed by a rapid bolus of 20 ml of saline. A computer-controlled syringe pump with fraction collector drew arterial blood samples at 3 sec intervals for the first min, and at 10 sec intervals for the second minute. Eight more samples were drawn manually up to 15 min after administration of the ICG bolus dose. The sampling system consists of a disposable sampling set, an automatic sampler and a carrousel with test tubes. The automatic sampler is a custom made device that can move a syringe plunger to a set volume and moves a stopcock in coordination with sampling. The sampler is connected to a computer that times the sampling. Because of limitations in blood flow in the radial artery the sampling rate is maximally 1 per 3 sec. The carrousel moves synchronous to the sampling. The disposable sampling system consists of a syringe with a stopcock, extension tubing between the patient and the sampling syringe, and extension tubing to the test tubes mounted on the carrousel. The extension tubing to the patient is connected with a stopcock to the intra-arterial catheter. The volumes in the extension tubes with the stopcocks have a dead space volume of 1.8 ml. The sampling
volume was set to 1.8 ml and is thus identical to the dead space in the
extension tubes. Each sampling cycle consisted of drawing the sample from the
patient, turning the stopcock on the syringe and then ejecting the content of the
syringe to the extension tube to the test tubes. Since the movement of blood in
the system is fully controlled, mixing of samples in the tubing is unlikely and
each sample represents the concentration at the sampling time. The blood
samples were collected in heparinized glass tubes and processed immediately.

The patients in whom the finger probe was used for determination of ICG
concentrations underwent up to 3 repeated measurements in the same session
separated by a time period of at least 15 min to allow for dye excretion. During
each measurement patients were awake and in a hemodynamically stable
condition. In the patients in whom the nose probe was used for the
determination of ICG concentrations, single measurements were performed, as
patients were studied during induction of anesthesia.

**Measurements of ICG in blood**

The concentrations of ICG were determined in whole blood using High
Performance Liquid Chromatography (HPLC) (Separations analytical
instruments, Hendrik-Ido-Ambacht, The Netherlands; column: Ultrasphere ODS
4.6 x 7.5 cm 244254, Beckman Coulter, Mijdrecht, The Netherlands) with
ultraviolet and fluorescence detection. For each patient a calibration curve was
constructed, using the patients own blood prior to injection of ICG. The
fluorescence settings were as follows; excitation at a wavelength of 780 nm
and the emission wavelength at 810 nm with a gain of 100. ICG was also
measured at 777 nm, its peak in the spectrum. Both ICG and its degradation
product were identified by a diode array (Photodiodedetector PDA 100, Dionex,
Amsterdam, The Netherlands). The detection limit of the whole blood assay for
ICG was at 0.15 or 0.2 mg.L⁻¹. The coefficient of variation was less than 6 %
over the range from 0.5 to 10.45 mg.L⁻¹.

**Data collection and processing**

Pulse Dye Densitometry was performed using the DDG-2001 A/K (Nihon
Kohden, Tokyo, Japan). Data were transferred to a laptop-computer and
imported into a spreadsheet program (Excel 2000, Microsoft Corporation,
Seattle, U.S.A.). Arterial blood concentrations were imported in the same
spreadsheet program to undergo further analysis.

Cardiac output was calculated by dividing the administered ICG dose by the
area under the first-pass concentration-time curve (A₁+A₂; see below). The
shape of the first pass concentration-time curve, including all data before evidence of ICG recirculation, was log-linearly described by the sum of two Erlang functions, each representing the convolution of $n$ 1-compartment models connected in series $^{17}$

$$
C(t) = A_1 \ast \frac{k_1^{n_1} t^{n_1-1}}{(n_1-1)!} + A_2 \ast \frac{k_2^{n_2} t^{n_2-1}}{(n_2-1)!}
$$

where $n_1$ and $n_2$ are the number of compartments in series in the central delay elements; $k_1$ and $k_2$ are the rate constants between the compartments in series; $n_1/k_1$ and $n_2/k_2$ are the mean transit times (MTT) of the central delay elements; $A_1$ and $A_2$ are the areas under the first pass concentration time curves. The two Erlang functions were fitted to the data using the solver function in Excel (Microsoft Corporation, Seattle, U.S.A.), whereby data were uniformly weighted.

Total blood volume was estimated as $TBV = D / C_0$, in which $D$ is the dose administered and $C_0$ is the log-linearly back-extrapolated concentration at mean transit time, when first mixing but no elimination of ICG has occurred during the first circulation. The intrathoracic or central blood volume is determined as the product of $CO$ and MTT.

**Statistical analysis**

Comparison of the two methods in both groups (nose and finger probe) was done by Bland-Altman analysis$^{18}$, reporting mean difference (bias) and Limits of Agreement (LOA, bias ± 2 SD). In the group using the finger probe, the total variance due to intra- and interindividual differences has been taken into account using additional one-way analysis of variance.$^{19}$ Comparison of ICG peak concentrations using the finger or nose probe versus the arterial blood ICG concentration was performed by a paired T-test (SPSS, version 14.0).

**Results**

**Patients**

Patient characteristics of both groups are represented in table 1.
Table 1 Patient characteristics (data presented as median and range).

<table>
<thead>
<tr>
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<th>Finger probe group</th>
<th>Nose probe group</th>
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</thead>
<tbody>
<tr>
<td>Patients (n)</td>
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<td>10</td>
</tr>
<tr>
<td>Sessions (n)</td>
<td>26</td>
<td>10</td>
</tr>
<tr>
<td>Gender (M/F)</td>
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<td>2 / 8</td>
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<td>Age (yr)</td>
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<td>Weight (kg)</td>
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<tr>
<td>Height (m)</td>
<td>1.70 (1.56-1.87)</td>
<td>1.71 (1.58-1.97)</td>
</tr>
<tr>
<td>Body mass index (kg m-2)</td>
<td>23.5 (21.2-29.3)</td>
<td>23.2 (18.9-25.1)</td>
</tr>
</tbody>
</table>

ICG concentration curves

In both sets of patients, it regularly proved difficult to obtain an adequate signal quality.

In two sessions performed with the finger probe the generated ICG curves consisted of a small-based concentration peak, generating CBV of 22.7 and 26.8 L and an accordingly high CO of 35 and 47.3 L.min⁻¹. Since these figures were far beyond physiologically acceptable values, the results for CO and CBV were excluded from the Bland-Altman analysis.

Due to the rapid arterial blood sampling it was possible to obtain an adequate number of data points to define the peak in the arterial ICG concentration, including recirculation of the dye. In each experiment, on average, 32 arterial blood samples for blood ICG concentration determination were taken, of which 20 were in the first minute. In both groups, the peak blood ICG concentration measured by PDD was generally higher than in the arterial blood. The average difference of +29% ± 36% (mean ± SD) using the finger probe (n=24, p<0.001) and +34.2% ± 46.5% (mean ± SD) using the nose probe (n=9, p=0.079). Furthermore, the peak concentration of ICG measured by PDD lagged behind the arterial blood ICG peak concentration. The average time shift between the noninvasively and invasively measured ICG concentration was 9.6 sec ± 9 (SD) in the finger probe group. In the nose probe group this time shift did not occur. Examples of ICG concentration curves showing a marked difference in MTT between invasive and noninvasive ICG measurements using the finger probe are shown in figure 1. The data were obtained from the same patient in
successive sessions. In this case no clear differences in peak concentrations were observed.

Figure 1 Indocyanine Green (ICG) concentration-time data of 3 sessions in a single patient, determined by the pulse dye densitometry (PDD) finger probe (open markers) and from arterial blood (closed markers). Note the time shift in the first experiment (panel A) and the difference in first circulation in the third experiment (panel C).
Hemodynamic parameters using the finger probe

In 24 datasets the cardiac output (CO) and central blood volume (CBV) and in 26 datasets total blood volume (TBV) were calculated and compared. TBV was recalculated from the raw data, using a spreadsheet with the algorithms as described in the methods section, to correct for major noise artifacts by adjusting the interval of the curve used for back-extrapolation to $C_0$. The Bland-Altman analysis revealed a mean absolute difference and limits of agreement between PDD and invasive measurements for CO of -0.43 L.min$^{-1}$ (-4.76 and 3.90 L.min$^{-1}$); for CBV of 0.76 L (-2.45 and 3.97 L) and for TBV of -1.42 L (-3.88 and 1.03 L) (Figure 2). The Bland-Altman analysis revealed a mean relative difference and LOA between PDD and invasive measurements for CO of -5% (-56% and 47%); for CBV of 21% (-54% and 96%) and for TBV of -15% (-38% and 8%).

Hemodynamic parameters using the nose probe

In all datasets (n = 10) the CO, central blood volume (CBV) and total blood volume (TBV) were compared. The Bland-Altman analysis revealed an absolute bias and LOA between PDD and invasive measurements for CO of 2.26 L.min$^{-1}$ (-4.67 and 9.20 L.min$^{-1}$); for CBV of 0.79 L (-2.08 and 3.66 L) and for TBV of 0.73 L (-3.48 and 2.01 L) (Figure 3). The Bland-Altman analysis revealed a mean relative difference and LOA between PDD and invasive measurements for CO of 30% (-67% and 127%); for CBV of 48% (-98% and 193%) and for TBV of -10% (-47% and 27%).

Discussion

Various studies on the comparison of PDD derived cardiovascular parameters versus intravascular measurements by e.g. a pulmonary artery catheter have been published: on cardiac output (CO)$^{3,20-23}$, central (or intrathoracic) blood volume (CBV)$^4$, total blood volume (TBV)$^{13}$, and hepatic blood flow.$^{24}$ The noninvasively derived dye dilution curve was not validated in any of these publications, yet the derived parameters were compared to results obtained by other methods of measurement. In other words, no study so far accurately compared frequently gathered arterial ICG concentrations before complete mixing had occurred with the noninvasive ICG data by PDD and evaluated the derived cardiovascular parameters based on these invasive and noninvasive ICG dilution methodologies.
Figure 2 The bias (PDD-arterial) and limits of agreement (± 2 SD) for cardiac output (A), central blood volume (B) and total blood volume (C), measured by the pulse dye densitometry (PDD) finger probe.
Figure 3 The bias (PDD-arterial) and limits of agreement (± 2 SD) for cardiac output (A), central blood volume (B) and total blood volume (C), measured by the pulse dye densitometry (PDD) nose probe.
In this study we evaluated the hemodynamic parameters based on noninvasive ICG measurements by pulse dye densitometry, using a finger or nose probe, and compared them to those based on invasive ICG measurements in the arterial blood.

Arterial measurement of ICG concentrations is not considered to be the gold standard for the determination of cardiovascular parameters. On the other hand, comparison of these two methods is the most accurate way to evaluate the underlying technique used in PDD. Hence, Bland-Altman analysis of the results is the only allowed method for comparison of the results. The data in the first part of this study were gathered using the finger probe; initially this probe was chosen because the anatomical proximity of the two sample sites (the radial artery and the index finger) was likely to improve the comparability of the data. To improve the comparison, we included a second patient group using the nose probe. For determination of TBV and the ICG plasma disappearance rate (ICG-PDR), both probes generally are considered equally reliable.\textsuperscript{5,6,14} Some studies, however, suggest that the nose probe may be more reliable for determination of cardiac output\textsuperscript{21}, whereas other studies do not favor either probe for CO determination.\textsuperscript{13,15} Our results show an overestimation of CO by the nose-probe and underestimation by the finger probe. Providing physiopathological support for this finding is difficult. One can speculate that the influence of vasoconstriction in the microvasculature, tissue volume at the probe site and the proportion of mixed tissue absorption in the digits is more elaborate than in the nasal wing.

We conclude that transcutaneous measurements by PDD result in higher (finger and nose probe) and postponed (finger probe) ICG concentrations compared to arterial ICG concentration measurements of the same bolus of ICG. Any difference in detection of ICG peak concentration has influence on both CO and CBV calculation, as it affects the AUC. The phase shift is of consequence in the calculation of CBV, since it directly affects the MTT. Therefore, the PDD-derived hemodynamic parameters cardiac output and central blood volumes are inaccurate. As a consequence of the wide limits of agreement, in an individual patient with a CO of 6 L.min\textsuperscript{-1}, PDD could measure a CO of 3 L.min\textsuperscript{-1} or 9 L.min\textsuperscript{-1}. This huge uncertainty significantly limits the use of PDD for the monitoring of CO in the individual patient.

The measurements of total blood volume correlated better. This can be explained by the fact that TBV is less influenced by a time shift in the first circulation curve, as long as the elimination curve compares well between the two methods. Before accepting a PDD generated TBV value, we recommend
taking the complete concentration curve into consideration: when (motion) artifacts occur in the interval used for back-extrapolation, estimation of the down slope may be highly affected and manual adjustment of the interval is necessary.

The clinical conditions, under which the two probes were tested, varied between subjects in the two studies. At higher ranges of the parameters determined, the relative differences between the methods did not show an increase. The magnitude of the bias is therefore not related to the range of cardiac output or TBV in our study.

**Study limitations**

Ideally, the comparison of the two probes would have been performed in a randomized cross-over manner, but due to the separate protocols and pre-operative character of the experiments, this was not possible. Unfortunately it was not possible to use both probes simultaneously.

Underestimation of the peak ICG concentration by arterial sampling may in part be explained by the lower sample frequency. However, even when smoothing of the curve by a moving average is assumed, the influence on the AUC should be minimal. Furthermore, the gathering of 6-8 arterial blood samples during the first circulation of ICG (usually 12-20 sec) is the maximum achievable in practice. Variability in the ICG measurements in blood was low; in some cases however, it was more difficult to fit the first circulation. This could occur in patients with a high cardiac output, generating less data points in the first circulation of the dye.

In our study two sessions performed with the finger probe were excluded in the Bland-Altman analysis for CO and CBV. The detected PDD concentration curves showed a small based peak during the first circulation, generating a very small AUC. The results for CO and CBV were over 20 L (per min) and clearly wrong. Incorrect detection of the PDD may lie in the signal:noise ratio being influenced by motion or low pulsatility of the signal due to constriction of the microvasculature in the digit. The signal may even be merely a reflection of a mixed tissue level, due to the passage of the indicator through small arterioles, capillaries and small veins. There was however no obvious reason for the findings in these sessions. Bremer et al. report an average of 5 performed measurements by PDD to collect 3 apparently valid recordings, Haruna and colleagues excluded 3 out of 10 volunteers due to motion artifacts and/or low signal:noise ratio. Secondly, in cases where the declining part of
the concentration peak is not smooth, the PDD device may generate an inadequate fit of the first circulation. This cannot be adjusted manually.

We recommend the (future) user of the PDD method to pay attention to the following aspects: optimize signal quality by guarding the temperature of the probe site, avoid vasoconstriction, avoid excess light at the probe site, avoid motion of the patient or probe, check the signal quality during the measurement (aim for a minimum of 2 out of 5 units on the display), observe the concentration curve for adequacy of fit of the first circulation, adjust the interval for back-extrapolation if necessary.

**Conclusion**

In conclusion, the results of this study significantly question the reliability of pulse dye densitometry by Nihon Kohden for cardiac output and central blood volume measurement in the individual patient. The nose and finger probe were equally unreliable. Given the wide limits of agreement, pulse dye densitometry could misinform the clinician about the actual hemodynamic status of the patient. Despite the need for less invasive methods of cardiac output measurement, better alternatives than PDD are required. PDD is better suitable for measurement of total blood volume, as our findings indicate. PDD is used also as an indicator for hepatic clearance and hepatic blood flow, especially during liver transplantation.\(^{24-26}\) The application of PDD for this purpose remains to be validated.

**References**


