Cardiac output and mixed venous \( \text{O}_2 \) content measurements by a tracer bolus method: animal validation study

JUSTIN S. CLARK, YUXIANG J. LIN, MICHAEL J. CRIDDLE, ROGER JONES, ERIK KOPPERT, AND PATRICK SWIER
Department of Biomedical Engineering and Medical Physics, LDS Hospital, Salt Lake City 84103; Department of Medical Informatics, University of Utah, Salt Lake City 84132; and Anesthesiology Department of Primary Children’s Medical Center, Salt Lake City, Utah 84113

Clark, Justin S., Yuxiang J. Lin, Michael J. Criddle, Roger Jones, Erik Koppert, and Patrick Swier. Cardiac output and mixed venous \( \text{O}_2 \) content measurements by a tracer bolus method: animal validation study. J. Appl. Physiol. 85(2):459–464, 1998.—A bolus method for noninvasive measurement of cardiac output (CO) and mixed venous oxygen content (\( C_{\text{vO}_2} \)) has been tested against absolute CO and \( C_{\text{vO}_2} \) standards in dogs. No statistical differences in CO were found between bolus method and electromagnetic flowmeter measurement comparisons in an 18-dog study in which CO varied from 0.5 to 3.0 l/min. The SD for all paired differences was 0.14 l/min; however, data averaging over 10-min intervals were found to reduce the CO measurement uncertainty to <0.08 l/min. The ability of the bolus method to follow rapid CO changes, experimentally produced by control of a pump (surgically placed between the superior and inferior vena cava and the right atrium), was documented and found to satisfy CO monitoring requirements of unstable subjects. \( C_{\text{vO}_2} \) bolus values were found to be statistically equivalent to reference measurements.

multiple inert gas; dogs

A NONINVASIVE, MULTIPLE INERT TRACER BOLUS method for near-continuous measurement of cardiac output (CO) and mixed venous oxygen content (\( C_{\text{vO}_2} \)) has been reported in a theory paper (2). By utilizing the airway as an inert-gas injection and measurement port, the method claims to provide unbiased measurements without the requirement of calibration from an external source. Absolute measurements are obtainable without the requirement of an airtight seal at the airway by utilization of bolus injection of tracer gases, synchronized with inspiration.

The major purpose of the validation study described in this paper was to test the method’s claim of nonbias over a wide range of CO values for a given size animal. Further purposes of this study were to demonstrate the degree to which the method can follow rapidly changing CO values and the ability of the method to measure \( C_{\text{vO}_2} \).

METHODS

Instrumentation and analytical procedures. The electromagnetic flowmeter (EMFM) was chosen as the standard for flow measurement, being blood flow calibratable to within 5% absolute. The EMFM (Statham model SP 2201) was calibrated volumetrically with saline and adjusted for blood according to the manufacturer’s recommendations. To provide positive control of CO, a pump was surgically placed between the central venous supply and the right heart as described in Fig. 1. The EMFM placed at the output of the pump provided the CO standard. An EMFM placed around the aorta (in some experiments) provided a relative CO waveform comparison to ensure that the waveform measured at the pump was representative of the CO response measured at the aorta.

A modified polarographic method (4) served as the \( C_{\text{vO}_2} \) standard to avoid 1) accuracy uncertainties of oximeter methods due to species differences in hemoglobin spectral characteristics and 2) problems finding a technician with up-to-date skills to reliably operate a Van Slyke apparatus. First-principle measurement status, equivalent to the Van Slyke manometric method, is achieved by automatic nulling technique, which eliminates both the influences of sensor characteristics and operator skill from the \( C_{\text{vO}_2} \) measurements. The bias and SD of the nulling apparatus (compared with the Van Slyke) has been reported to be 0.006 and 0.35 vol%, respectively (4).

To compensate for the deficiencies (primarily instability) of the quadrupole mass spectrometer (Balzers QMA 140), an automatic calibration unit was used as illustrated in Fig. 2. Because a two-stage gas-inlet system requires calibration gas at the tip of the inlet, such was provided by the configuration illustrated in Fig. 2A, which allowed the inlet to receive calibration gas from either of the two calibration sources (cal gas 1 and cal gas 2, respectively) or airway gas when both calibration sources are closed. Calibration flow was adjusted to exceed by ~20% the analyzer inlet flow rate. Valving for the two calibration sources and tracer-gas injection are shown in Fig. 2B. Note that the analyzer inlet is upstream from the tracer gas-injection port, preventing the possibility of analyzer saturation from direct sampling of the bolus.

The gas composition of cal gas 1 consisted of pure nitrogen, thus providing a zero calibration for all analyzer channels. The gas composition of cal gas 2 was a variable mixture of nitrogen, oxygen, carbon dioxide, and the inert tracers. The tracers were sulfurhexafluoride [blood-gas partition coefficient (\( \alpha \)) = 0], methylvinylether (\( \alpha = 2.7 \)), and dimethylether (\( \alpha = 11.1 \)). Concentration control was provided by a four-channel version of the Corning 192 precision gas mixer developed by this group (1). The tracer input source to the mixer was the tracer gas tank, which supplied the tracer-injection valve (Fig. 2B). Gas mixer control resided in the host microprocessor, which also controlled the mass spectrometer and injector, as illustrated in the block diagram of Fig. 3. A breath sensor (thermister) provided injector-timing information, which was input to the host microprocessor. The host microprocessor also received data from the EMFM. Cal gas 2 concentrations of oxygen and carbon dioxide were automatically adjusted to closely match the expired concentrations of these respective gases, thus removing linearity concerns primarily associated with oxygen. The range of the tracer mix was adjusted to provide cal gas 2 tracer concentrations close to those in the expired gas, which, in addition to ranging, allowed the tracer calibration and injection sources to be common with no requirement for preanalysis.
Bolus volume calibration was accomplished volumetrically by using a free-sliding 10-ml glass syringe. Average \( \lambda \) values were determined by using a blood solubility measurement apparatus (5) developed by this group.

Arterial \( P_{O_2} \), arterial \( P_{CO_2} \), pH, arterial oxygen saturation (\( S_{aO_2} \)) and hemoglobin (Hb) measurements were provided by the clinical laboratory of the Primary Children’s Medical Center.

Animal preparation. Mongrel dogs ranging in weight between 18 and 22 kg were used for the CO studies. The dogs first received pentobarbital sodium (30 mg/kg iv). Further doses of pentobarbital sodium were given during the experiment to maintain an absent corneal reflex. After intubation and connection to the ventilator, dogs received scopolamine (0.2 mg initially, then 0.1 mg/h) and morphine (1.5 mg). Before incisions, dogs were injected subcutaneously with lidocaine (0.5%). An arterial line was placed in the femoral artery for blood pressure and blood-gas monitoring. Venous lines were placed in the left and right femoral vein for administration of fluids and drugs and central venous pressure monitoring. To reverse the dehydrated state, each dog received 1.5–2 liters of fluid before surgery. Maintaining blood pressure and venous tone at desired levels was achieved by a Levophed and/or a Neosynephrin drip (1 or 2 vials per 500 ml of fluid). Arrhythmias (occurring infrequently) were treated with lidocaine (1%) and calcium chloride.

To gain access to the chest, an incision was made at the fourth intercostal space on the right side, along the superior rim of the fifth rib. The subcostal muscles and part of the back musculature were dissected, perforators ligated, and hemostasis was achieved. For opening of the pleural cavity, the incision was extended as far as possible toward the sternum, without damaging the internal mammary artery. A ribspreader was inserted and, with the lungs held aside by using
wet gauze, exposure of the heart was obtained. At regular intervals, the lungs were slightly overinflated to prevent atelectasis.

Vessel loops were used to control (after preparation) the superior vena cava (SVC), the azygos vein, and the inferior vena cava (IVC). The aorta was prepared to fit the electromagnetic flowmeter cuff (for those experiments designed to test CO waveform performance of the electromagnetic flowmeter located at the pump). After the pericardium was opened, four purse-string sutures (Prolene 2.0) were put in the heart: at the junctions of the right atrium and the SVC, at the junctions of the right atrium and the IVC, the anterior wall of the right ventricle, and in the auricle of the right atrium. The azygos vein was then ligated, and the animal received its initial dose of heparin (500 units/kg) as a preparation for the bypass procedure.

Two right-angled uptake catheters were then inserted through the right atrial wall taking up blood from the SVC and IVC. One straight-return cannula was inserted through the anterior wall of the right ventricle, with the tip of the cannula just below the pulmonary valve. The uptake cannulas were then connected to the tubes leading to the bypass pump, without letting air into the tubes. The return cannula was concurrently connected to the return tube from the bypass pump. The coronary-flow-uptake cannula was inserted through the purse-string suture in the auricle of the right atrium. The vessel loops (umbilical tape) around the SVC and IVC were then tied against the cannulas, so that all blood was forced through the cannulas to the bypass pump.

A bolus of nembutal was given by intravenous injection to kill the animal at the end of the experiment.

To perform the \( CV_O_2 \) validation studies, central venous catheters were placed in the pulmonary arteries of mongrel dogs for which no open-chest surgical procedures had been performed. Of nine such dogs, one was doubly cannulated, as described in the theory paper (2), to permit one lobe set to dynamically control the \( CV_O_2 \) while the other set provided bolus-method \( CV_O_2 \) measurement opportunities. The remaining eight were cannulated with standard endotracheal tubes.

Experimental procedures. Because of the necessity of performing the CO studies under open-chest conditions, special procedures were required to correct for tracer-gas diffusion loss into the atmosphere via the lung tissue. At the conclusion of each open-chest experiment, the pump was turned off to provide a zero CO calibration point and allow the diffusion-loss calculation to be made for the tracer gases. To test the bolus method assumption that such diffusion loss from lung tissue to the chest wall is insignificant, two closed-chest bolus-method experiments were performed on dogs that had no chest surgery. At the conclusion of these experiments, the heart was suddenly and irreversibly stopped by nembutal injection while a constant ventilation and continuing bolus measurements were maintained at a confirmed CO of zero.

A standard experimental protocol with the extracorporeal pump control shown in Fig. 1 called for adjusting the pump to reach maximal CO values that could be maintained by the dog in a good physiological steady state. The pump would then be suddenly adjusted to various lower levels and returned to the higher control value. However, in most cases, loss of venous tone and subsequent collapse of the SVC and IVC were the limiting factors in establishing control CO values, despite extensive use of vasoconstrictive drugs. Therefore, events outside of experimental control governed the CO values while experimental efforts were directed toward dog’s survival. Fortunately, such instability did not interfere significantly with achievement of the experimental goals of testing the bias and stability of the bolus method under rapidly varying CO conditions.

A total of 18 dog experiments were performed in which comparisons between the bolus method and extracorporeal EMFM measurements were made. In most cases, the number of comparisons was determined by the length of time the animal survived, which created a large variability in the number of comparisons per dog study. For the first 12 dog studies, gas-analyzer calibrations were performed every 2 h and/or immediately after the death of the animal. For the last six experiments, the calibration procedure was modified to be applied automatically and breath by breath during alternate inspiratory phases of the respiratory cycle. In all studies, the ventilator (Hamilton model 608) rate was 12 breaths/min; bolus delivery occurred early in inspiratory flow in a cycle of 16 consecutive breaths followed by an equal number of
nonbolus breaths. The estimated equilibrium method for ventilation-perfusion ratio (V/Q) extraction for three tracer gases was used as described in the theory paper (2).

To perform the $CvO_2$ studies, six of the intact dogs with standard endotracheal tubes were paralyzed and ventilated with the Harvard animal respirator. Two were placed on the Bird pressure ventilator (Mark B) and allowed to control the ventilator. Each measurement in a study consisted of ~15 min of gas data collection, with mixed venous blood samples obtained at the beginning and end of the 15-min segment. The oxygen content of the blood samples was determined by the automatic oxygen-content analyzer (4) in triplicate, using up most of the 2-ml blood sample to reduce the risk of measurement error due to red blood cells and plasma separation. The average of the in vitro $CvO_2$ measurements was compared with the average bolus $CvO_2$ measurement obtained over the 15-min interval. This procedure was repeated after at least 1 h had transpired, giving the animal the opportunity to change states.

A doubly cannulated dog was used to demonstrate the ability of the bolus method to follow dramatic changes in $CvO_2$ brought about by transiently supplying the left lung with 100% nitrogen for 90 min and then returning the left lung inspiratory supply to room air. Bolus $CvO_2$ measurements were performed in both sets of lobes during room-air breathing. Bolus $CvO_2$ measurements were restricted to the right lobe set when nitrogen was being supplied to the left lobe set.

An average of ~4 bolus-method and reference $CvO_2$ comparisons was obtained per dog, giving a total of 34 measurement pairs for the 9-dog study.

Data analysis. Interdog bias and range bias of the bolus method, referenced to the EMFM standard, were analyzed as follows. Interdog bias is represented by the vertical distance between data points, represented by average bolus CO and corresponding average EMFM CO for an individual dog study, and the all-dog linear regression line. Range bias was determined by first grouping the all-dog data into three ranges of CO values. Bias for each range is then represented by the vertical distance between average data points, represented by the average bolus CO and EMFM CO corresponding to these individual groupings, and the all-dog linear regression line.

Statistical significance of the CO and $CvO_2$ calculation biases was determined by the t-test at 95% confidence limits.

RESULTS

CO measurements. The all-dog paired CO differences (bolus - EMFM), consisting of 293 paired measurements for 18 dogs, are plotted in Fig. 4 against the average of the bolus and EMFM CO measurements. The top and bottom dotted lines represent $+2 \text{ SD}$ and $-2 \text{ SD}$, respectively, about the all-dog mean of paired differences. The all-dog mean (bias) and SD are 0.03 and 0.14 l/min, respectively.

Ten-minute averages of the all-dog paired CO differences are presented in Fig. 5. Note the reduction in SD to 0.08 l/min and the marked reduction in outliers.

The interdog bias values are represented in Fig. 6 by the vertical distances between the individual dog CO averages and the all-dog regression line. The slope of the all-dog regression line ($y = 1.02x - 0.01$) is not statistically different from 1.

![Fig. 4. All-dog paired cardiac output (CO) differences (ΔCO) (bolus – EMFM) plotted against average (Ave) of bolus and EMFM CO for each paired measurement. Top and bottom dotted lines represent $+2 \text{ SD}$ and $-2 \text{ SD}$, respectively, about the all-dog mean of paired differences.](image1)

![Fig. 5. 10-min data averages of the all-dog paired CO differences (bolus – EMFM) plotted against average of bolus and EMFM CO measurements for each 10-min interval. Top and bottom dotted lines represent the 10-min $+2 \text{ SD}$ and $-2 \text{ SD}$, respectively, about the all-dog mean of paired differences.](image2)

![Fig. 6. Interdog bolus CO average plotted against corresponding EMFM CO average for each of 18 dogs. Solid line represents the all-dog linear regression given by $y = 1.02x - 0.01$. Interdog bias for each dog is represented by vertical distance from its point on the graph to the regression line. Of 18 bias values, 3 (▲) were statistically different from 0.](image3)
significantly different from unity, as it is within the uncertainty of the EMFM calibration. The intercept is also insignificant, consistent with the results of the two closed-chest experiments that showed no measurable zero CO bias.

Of the 18 dogs, only 3 had biases that were statistically significant from 0 as represented by the symbols. The interdog SD (0.07) is little different from the 10-min SD, suggesting that a significant amount of the 10-min average variance shown in Fig. 5 is due to interdog bias.

The range bias data (presented in Fig. 7) are the all-dog averages corresponding to CO ranges of 0.0–0.5, 0.51–1.5, and 1.51–3.0 l/min. The range bias values, given by the vertical distances between the average value in each range and the all-dog regression line, are not significantly different from zero.

Representative examples of the ability of the bolus method to follow changes are illustrated in Fig. 8.

$C_vO_2$ measurements. The paired $C_vO_2$ differences (bolus – in vitro), consisting of 34 paired measurements for 9 dogs, are plotted in Fig. 9 against the average of the bolus and in vitro $C_vO_2$ measurements.

The top and bottom dotted lines represent the $+2$ SD and $-2$ SD, respectively, about the mean of the 34 paired differences.

The results of the double-cannulation transient study, presented in Fig. 10, demonstrate the ability of the bolus method to follow transients and to measure $C_vO_2$ from either lobe set when alveolar gas values are within physiologically compatible limits.

**DISCUSSION**

The CO results of this study demonstrate that by analysis of multiple inert gases applied noninvasively at the airway, absolute measurements of CO are obtainable in subjects with moderately abnormal lungs, assuming that $S_aO_2$ and Hb are independently obtainable. The first significant result is the close identity between the bolus CO calculations and the EMFM standard for the total dog set throughout the range of CO comparisons, particularly when data are averaged for 10-min
intervals. It is also worthy to note that the apparent lack of significant bias in bolus CO measurements was obtained with lungs having nonuniformity of V/Q sufficient to cause bias errors averaging 19%, had a single-compartment rather than a three-compartment model been applied to the data. (This estimate was obtained by withholding the dimethylether and SaO2 data from the CO analyses.) Whereas these results cannot be extrapolated to predict bolus measurement bias in subjects having a greater degree of V/Q nonuniformity, it is clear that V/Q correction did take place in the dogs of this study. Furthermore, these results are consistent with the simulation results presented in Table 4 of the theory paper (2).

The second significant result is verification of sufficient stability of the bolus method to enable the following of trends in CO in the presence of induced cardiopulmonary instability. However, it is clear from the experimental data (see Fig. 8) that induced cardiopulmonary instability added significantly to the apparent noise of the bolus measurements.

Instability of the quadrupole mass spectrometer proved to be a second significant source of noise. The employment of breath-to-breath calibrations, employed in the last six dog studies, produced a reduced SD of 0.10 l/min, compared with the all-dog SD of 0.14 l/min. Because the bolus method becomes increasingly sensitive to differential noise (noise between channels) as CO is reduced, this noise source explains much of the increase in percent noise with reducing CO. [This noise source is essentially absent if a magnetic sector mass spectrometer is employed as the gas analyzer (6).]

The interdog bias constitutes a third category of error. Whereas this bias appears as systematic error for a given dog study, it adds to the random noise of the all-dog data set. The most likely major source of this error is interdog variance of the inert-gas blood solubilities. As explained in METHODS, average blood solubility measurements from previous dog studies were used, since our solubility measurement apparatus was nonfunctional. The SD of this noise source (0.07 l/min) represents the limitation in SD reduction of the all-dog data achievable by averaging data for particular time segments. Therefore, the 10-min average SD of 0.08 l/min should be close to the lowest SD achievable from the all-dog data set. However, significant reduction SD in future studies should be attainable by measurement of inert-gas blood solubility values in individual subjects.

As described above, for the bolus method to provide an unbiased CO measurement, unbiased measurements of SaO2 and Hb are required. Noninvasive estimates of SaO2 are available through pulse oximetry; however, such noninvasive estimates of SaO2 measurement is not truly unbiased as it tends to estimate low in critically ill patients with compromised cutaneous circulation (3). Such appears to be a temporary problem, as methods to eliminate this bias are being developed with apparent success (7).

On the other hand, bolus CVo2 measurements, which are byproducts of the CO calculations, are not affected by the accuracy of the SaO2 estimate. The bolus CVo2 measurements show negligible bias when compared against the reference. Of the 1.3 vol% (2 SD) uncertainty represented in Fig. 9, no more than 0.2 vol% can be attributed to the reference [which has a 2-SD measurement uncertainty of <0.70% (4)]. This leaves a per-measurement 2-SD uncertainty estimate of 1.1 vol% and a 10-min average 2-SD uncertainty estimate of 0.69 vol% for the bolus method. The latter value represents <5% of the average CVo2 value. Much of this nonsystematic uncertainty (3.5%) can be explained by the dependency of the CVo2 calculation (1) on V/Q, when using the 10-min CO error data of Fig. 5. The remaining 1.5% is explainable from the quadrupole measurement uncertainty for inspired and expired oxygen (~4% relative).

For a clinical perspective, calculations of CVo2 based on Hb and mixed venous oxygen saturation measurements also have uncertainties that are probably no better than 5%. For example, precision of blood dilution limits the accuracy of Hb measurements to ~5% in instruments used in blood-gas laboratories (unpublished data from the Blood Gas Proficiency Committee of the American Thoracic Society, of which J. Clark is a member). Red cell settling, followed by inadequate mixing before measurement, represents another potentially significant (but as-yet-unstudied) source of CVo2 measurement error in the clinical laboratory. It would therefore appear that the bolus CVo2 measurement could compete well with laboratory CVo2 measurements in terms of accuracy when 10 min of data are used in the bolus CVo2 calculation.

This work was supported by the National Heart, Lung, and Blood Institute and by the Deseret Foundation.

Address for reprint requests: J. S. Clark, 825 N. 300 W., Suite 420, Salt Lake City, UT 84103-1414.

Received 10 October 1996; accepted in final form 26 March 1998.

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