



Karolinska Institutet

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Sektionen för Anestesiologi och Intensivvård

Capnodynamic determination of effective pulmonary blood flow and end expiratory lung volume Studies in children and paediatric animal models

Akademisk Avhandling

som för avläggande av medicine doktorexamen vid Karolinska Institutet offentligent försvaras i J3:06 Ulf von Euler, Nya Karolinska Sjukhuset, Solna

Fredagen den 5 April, 2019, kl 9.00

av

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Cover: An image of the lung vascular tree produced with synchrotron particle accelerator at The European Synchrotron Radiation Facility in Grenoble, France during an experiment we conducted there in 2017. The vascular tree shown includes the area close to the capillary vessels, where the gas exchange that the capnodynamic calculations are based on, takes place. Image produced by Broche L, 2017.

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Published by Karolinska Institutet

Layout Ulf Johansson

Printed by E-print AB 2019

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ISBN 978-91-7831-338-9

To Åke, Linus, Emma and Malin

***It ain't what you don't know that gets you into trouble.
It's what you know for sure that just ain't so.***

The origin of this quote is in doubt. It is variously attributed to Mark Twain or Josh Billing but in reality, its origin is unknown.

ABSTRACT

Dynamic capnography is a haemodynamic and respiratory monitoring technique enabling continuous cardiac output (Effective Pulmonary Blood Flow, CO_{EPBF}) and end expiratory lung volume (Effective Lung Volume, ELV) assessment in mechanically ventilated subjects. The method utilizes variations in exhaled CO_2 , implemented via periods of deliberate changes in the respiratory pattern. The method has been validated against high precision reference methods in animal experiments. Data from these studies have pointed to a potential use also in children, a patient group suffering from the lack of reliable and easy accessible cardiac output/functional residual capacity (CO/FRC) monitoring methods. We have validated dynamic capnography in a series of paediatric clinical studies and experimental models.

CO_{EPBF} was tested for agreement of absolute values and ability to detect change against suprasternal Doppler, CO_{SSD} , in anaesthetized children and against transpulmonary flow probe, CO_{TS} , in a porcine model mimicking the clinical study. CO_{EPBF} was also tested for the same qualities, against CO_{TS} and CO_2 Fick (CO_{Fick}) in a model of hypoxia induced pulmonary hypertension in piglets. The respiratory parameter ELV was examined for consistency against helium wash out in a paediatric rabbit model of CO_2 -induced pneumoperitoneum. In addition to this, the same protocol was used to determine the PEEP level associated with most favorable conditions for preservation of lung homogeneity and CO_2 clearance.

CO_{EPBF} showed good agreement and trending ability when compared to CO_{TS} and CO_{Fick} in the experimental setting. In the clinical study, CO_{EPBF} performed in the expected way. The reference method CO_{SSD} exhibited operator dependent qualities and appeared less reliable than CO_{EPBF} . Absolute values and changes in FRC could be monitored adequately by ELV, provided the application an adequate PEEP. The adequate PEEP level was also associated with optimal preservation lung homogeneity and CO_2 removal, thus suggesting the use higher level of PEEP during laparoscopy.

Dynamic capnography appears to be a reliable and accurate method for continuous CO and FRC monitoring and is a promising concept for future studies.

Keywords: cardiac output; children; carbon dioxide; measurement; ultrasonic; hypertension, pulmonary; carbon dioxide; laparoscopy; PEEP; functional residual capacity

LIST OF PUBLICATIONS

- I **Validation of capnodynamic determination of cardiac output by measuring effective pulmonary blood flow: a study in anaesthetised children and piglets**
Karlsson J, Winberg P, Scarr B, Lönnqvist PA, Neovius E, Wallin M, Hallbäck M
Br J Anaesth. 2018;121(3):550-8
- II **Capnodynamic determination of cardiac output in hypoxia-induced pulmonary hypertension in pigs**
Karlsson J, Wallin M, Hallbäck M and Lonnqvist P-A
Br J Anaesth 2018. Article in press
- III **Determination of optimal positive end expiratory pressure level for adequate lung homogeneity and CO₂ removal during laparoscopic surgery in an infant experimental model: a randomized investigation**
Karlsson J, ds Rocha A, Fodor H G, Lin N, Habre W, Wallin M, Hallbäck M, Lonnqvist P-A
Submitted

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LIST OF ABBREVIATIONS

BTPS	Body Temperature and Pressure Saturated	LCI	Lung clearance index
CO ₂	Carbondioxide	LoA	Limits of agreement
C _a CO ₂	Arterial CO ₂ content	LSC	Least significant change
C _C CO ₂	Pulmonary end capillary CO ₂ content	MAP	Mean arterial pressure
C _V CO ₂	Mixed venous CO ₂ content	MPE	Mean percentage error
CO	Cardiac output	n	Current breath
CO _{EPBF}	Cardiac output derived from EPBF	n-1	Previous breath
CO _{SSD}	Cardiac Output assed with suprasternal Doppler	PAC	Pulmonary artery catheter
CO _{TS}	Cardiac Output assed with Transsonic Flow Probe	PECO ₂	Partial pressure of end tidal CO ₂
CO _{Fick}	Cardiac Output derived from direct CO ₂ Fick	PEEP	Positive end expiratory pressure
CT	Computed tomography	PVR	Pulmonary vascular resistance
CV	Coefficient of variation	Q	Flow
Δtn	Current breath cycle time	p _a O ₂	Systemic arterial oxygen pressure
DC	Dynamic capnography	p _a CO ₂	Systemic arterial carbondioxide pressure
EELV	End expiratory lung volume	SCO ₂	Solubility coefficient for CO ₂ in blood
ELV	Effective lung volume	SD	Standard deviation of the mean
EPBF	Effective pulmonary blood flow	SF6	Sulfur hexafluoroide
FACO ₂	Alveolar CO ₂ fraction	TV	Tidal volume
F _i O ₂	Inspired oxygen fraction	VCO ₂	CO ₂ excretion
FRC	Functional residual capacity	VT _{CO₂}	Volume of CO ₂ eliminated by the current, nth, breath
He	Helium	VTI	Velocity time integral
iNO	Inhaled nitric oxide		

1 INTRODUCTION

The introduction of anaesthesia for surgical procedures is undoubtedly one of the most important additions to modern medicine when it was first initiated in the mid 19th century. Anaesthesia has since then been the subject of a steady development with increasingly advanced and sophisticated treatment and monitoring options, all driven by the desire to provide safe and optimal care for the patient.

The routine monitoring that is now considered mandatory during general anaesthesia offers continuous updates of physiological processes, which the early pioneers of anaesthesia could only dream about. At present, we can monitor a plethora of such processes of more or less decisive character. However, despite that it has been more than a hundred years ago since the introduction of modern anaesthesia, some of the more vital physiological events are still difficult to follow in a reliable way.

Cardiac output, the volume of blood pumped by the heart per minute, and functional residual capacity, the lung volume which is most important for gas exchange, are two examples of such processes. To be able to follow these parameters continuously and effortlessly must be regarded as one of the more sought after monitoring concepts in anaesthesia. Until recently there has been no readily available cardiac output or functional residual capacity monitoring method for children. The promising development of carbon dioxide-based cardiac output monitoring methods, ie Dynamic Capnography, in animals and adults has however opened up for this monitoring strategy also in children.

One of the purposes of all monitoring equipment is to provide the practitioner with an information basis upon which decisions can be made, treatment given or renounced. This places extremely high demands on the reliability and precision of the method. This is especially true in paediatric anaesthesia, where the margin for error can be small.

Added to this, methodologies should preferably be user-independent, require a minimum of education, be devoid of drift and recalibration and not requiring additional fluid administration.

The purpose of this thesis is therefore to provide a background of validation

and principle of cardiac output and functional residual capacity monitoring systems in children with special emphasis on Dynamic Capnography and to present a structured validation of Dynamic Capnography as a monitoring model in clinical and experimental paediatric studies.

2 BACKGROUND

During anaesthesia and surgery it is important to continuously monitor the physiological status of the patient and this is particularly true for haemodynamic and respiratory parameters.¹ It is therefore desirable to be able to measure or at least estimate the patient's cardiac output (CO) and functional residual capacity (FRC). In contrast to adult patients, where CO estimation using pulmonary artery catheter (PAC) thermodilution and complex tracer gas methods for FRC measurement, are accepted as "gold standards", these techniques are not routinely used in paediatric practice. This is mainly due to their cumbersome nature and increased risk of complications and these techniques are, thus, not suitable for continuous monitoring.²

For perioperative CO monitoring, the development of new methods have made the requirement for structured validations all the more crucial.³ Among these newer technologies are transpulmonary dilution techniques, Doppler measurements, arterial pressure curve-based CO monitoring and dynamic capnography (DC).⁴ Some, but far from all, of these technologies are properly validated for CO monitoring in children.⁵

The same applies for perioperative FRC monitoring where a limited number of methods are available.⁶ The accuracy of these methods have been tested in adults but as for CO monitoring, results based on adult patient studies cannot be directly extrapolated to paediatric practice, where the validation status for intraoperative FRC monitoring devices currently is insufficient.⁷

Since the majority of the CO and FRC monitoring options for paediatric care are intermittent, invasive, difficult to perform, needs recalibration and can not be used in newborn or small infants, the development a minimally-invasive, continuous, reasonably accurate and easy-to-use method would represent a seminal advancement of anaesthesia monitoring that would help guide therapy and most likely results in substantial benefits for many patient groups.⁸ One example of a method that potentially is associated with some these features is DC. DC is a methodology that determines changes in central haemodynamics and can also be used to estimate FRC but this methodology has not been possible to adapt to clinical use, until recently. DC uses modest predetermined variations of the respiratory pattern delivered by the ventilator

and combines this with high-precision, fast response, mainstream capnography and advanced mathematics to generate continuous breath-by-breath calculations of Effective Pulmonary Blood Flow (EPBF) and Effective Lung Volume (ELV). In a situation without major lung atelectasis EPBF has been found to closely equal CO.⁹

ELV represents the volume of the lung that takes part in actual gas exchange and is as such not a strictly defined anatomical volume and is therefore not exactly the same as FRC. However, ELV is very closely related to FRC and can be used as a proxy marker for FRC in the majority of scenarios.^{10,11}

This summary will give an overview on the background and rationale for CO₂ based CO and FRC measurement techniques in children and will focus more in detail on the development, validation and potential clinical usefulness of dynamic capnography in paediatric anaesthetic practice.

Clinical relevance of CO monitoring in children

One of the main advantages of continuous CO monitoring is the possibility of early detection of deviations in circulatory status.¹² This is of special importance when anaesthetizing small children where the margins for errors are small.¹³ Deviations in circulatory stability in children are today primarily detected through change of heart rate and blood pressure, two methods where changes often occur late in the process and have relatively low specificity for an approaching circulatory collapse. Changes in heart rate and blood pressure may be caused by factors other than failing circulation, for example, by non-optimal dosing of anaesthetics.

Looking ahead, there are studies indicating that the amount of intravenous fluid given under anaesthesia can be guided with help from CO monitoring by so-called stroke volume optimization.¹⁴ One can thus give the patient a tailor-made amount of fluid needed for the heart and other organs to function optimally, without risking giving too much or too little fluid. Currently, fluid administration in children is not individually adjusted but based on standardized values calculated from the patient's weight and metabolic need, which may increase the risk of improper amounts of fluids being given, with risk of fluid overload and hyponatremia.¹⁵ By continuously measuring CO, this risk could potentially be reduced. Furthermore, reliable information of CO is of great value when titrating inotropic and vasoactive drugs.

CO monitoring thus provides the information necessary to choose between appropriate actions (or no actions) and monitor the response of the interventions. For these reasons, estimation of CO can be regarded as an

important part in the haemodynamics assessment of the vast majority of anaesthetized children.

FRC and the concept of end expiratory lung volume (EELV)

Mechanical ventilation often leads to loss of static lung volumes and formation of atelectasis with subsequent impaired ventilation.¹⁶ This can cause both intra- and postoperative complications.¹⁷ To counteract this, new concepts based on open lung ventilation strategies with recruitment maneuvers and positive end expiratory pressure titration and have emerged.¹⁸ Common to the majority of these strategies is the goal to ventilate around the patients FRC.¹⁹ Strictly speaking, FRC refers to the lung volume at the end of expiration in a spontaneously breathing subject without adding positive end expiratory pressure (PEEP). During mechanical ventilation the term end expiratory lung volume is often used and refers to the volume at the end of expiration when PEEP is applied. In practice however, both terms are used alternately with the same meaning.

There is currently a large body of evidence indicating that mechanical ventilation is most effective and least damaging if performed around FRC, both in the normal and diseased lungs (e.g. acute lung injury).²⁰ Ventilation around the patients FRC has also beneficial effects on the interaction between pulmonary vascular resistance and pulmonary blood flow.^{21,22} Ventilation around FRC generally offers the lowest possible PVR and thus provides for optimal pulmonary blood flow and ventilation/perfusion conditions, as illustrated in *figure 1* below.

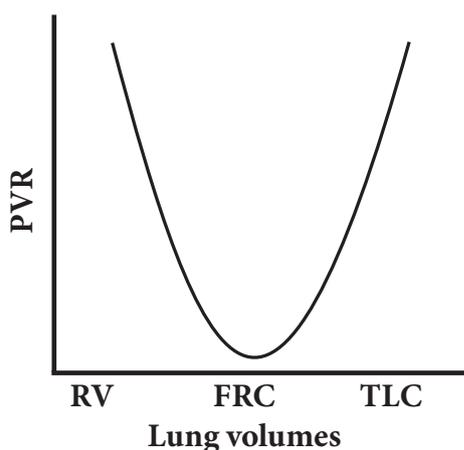


Figure 1. The relationship between PVR and lung volume.

RV: Residual Volume, TLC: Total lung capacity, PVR: Pulmonary vascular resistance

Modified from West JB: Respiratory physiology—the essentials, ed 9, Baltimore, 2012, Lippincott Williams & Wilkins.²³

Given this, it would be of great value to find an easy-to-use methodology that would help identify FRC, or something closely related to FRC. By doing this, it would improve the chance of providing a lung-protective ventilation strategy during anaesthesia and surgery, both in adults and children.¹⁶

To attempt to quantify FRC during anaesthesia is currently not routine clinical practice in adults or children since the available methods are too expensive and complex in the clinical context.²⁴ The capnodynamic method for determination of effective lung volume however has the promise to estimate continuously EELV and pulmonary blood flow, the combination of which provide valuable assistance for the clinician in the haemodynamic and ventilation strategies during anaesthesia of children.

Aspects on how to validate new CO and FRC monitoring techniques in children

In order to properly validate new measurements techniques, the most common rationale is to compare their performance against known reference standards. The results are then subjected to a standardized evaluation system consisting of a number of statistical tests.²⁵ There is currently an established structure on how to perform CO validation studies and it is generally recommended to follow this as far as possible, which we have had the ambition to do in the papers presented in this thesis. The structure defines suitable arrangements and statistical methods and is of great value for anyone involved in CO validation studies.

It's important to emphasize that regardless of the choice and quality of the statistical methods used, the main problem with CO validation studies, ie the lack of a true gold standard, is still hard to get navigate.

Statistical methods used in validation studies

The statistical methods often used and their limitations are explained in detail below.

Inherent precision

Defined as two times the coefficients of variation (CV) where $CV = SD/mean$ (SD=standard deviation).²⁶ It is important to report the precision of the reference method and the tested method since this partly defines the limits of acceptance of conformity between the methods. Inherent precision is also important when calculating the least significant change (LSC) as described in further detail below.

Correlation Coefficient

Correlation coefficients, for example using Pearson correlation or Spearman, for assessing agreement between a tested method and a reference was frequently used on early cardiac output validation studies and is still applied to

some extent in modern studies. Correlation coefficients shows the strength of the relationship between two measured variables and is an excellent indicator of this.²⁷ However, one of the limitations with this method is that correlation only shows the strength of the linear relationship. It really says very little about the actual agreement of absolute values between the methods. This limitation has made the method less its popular in modern CO validation studies despite still being very useful to show a correlation. However, if correlation analysis is to be used however, presenting the coefficient with associated 95% confidence interval is probably advisable since this gives far more information than just presenting a singel correlation value.

Agreement and Bias

Agreement between simultaneously recorded values of a tested method and a reference method are generally evaluated using Bland Altman analysis (28). One of the advantages of Bland Altman analysis is that it makes visual assessment of the agreement easy. The difference between the paired measurements is plotted against the average of the measurements. *Figure 2* below shows an example of a Bland-Altman plot from study II.

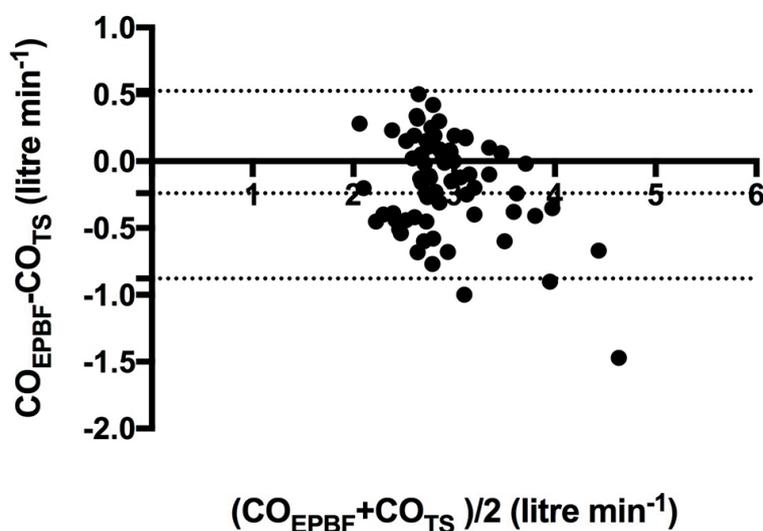


Figure 2. Example of a Bland Altman plot from study II. Black dotted lines represent bias and upper and lower limits of agreement.

An interesting topic is whether the Bland Altman analysis should be corrected for repeated measures.²⁹ In our studies we have not corrected for repeated measurements, given that each measurments is performed in a new situation where the reference value varies in a minor to moderate manner and thus represents independent data. Consequently, this represents serial and not repeated measurements.^{30,31}

The main difference if correction for repeated measures are applied however, is wider limits of agreement.

Bias and limits of agreement

Bias is defined as the mean difference between the tested method and the reference method in question. The spread of the included data points are presented as limits of agreement (bias \pm (1.96xSD)). One potential problem when interpreting the bias is that although the bias is small, the limits of agreement are often wide^{32,33,34} There is no general consensus on how wide the limits of agreement can be for the method to be accepted as clinically useful.²⁵ Unfortunately, the acceptable limits of agreement instead has to be defined on clinical judgment.

Bland-Altman analysis shows the overall bias and limits of agreement between two methods with the assumption that the differences between the compared methods are normally distributed.²⁸ However, the bias and limits of agreements may vary depending on the haemodynamic situation. If the haemodynamic situation is at the extreme of the spectra, the bias and LoA may very well be different and if possible, bias and limits of agreement should ideally be presented for each haemodynamic state³⁵ to overcome this problem.

Mean percentage error (MPE)

Defined as 1.96 x SD of the difference between the tested techniques divided by the mean of the reference technique as suggested by Critchley.³⁶ A mean percentage error less or equal to 30% is usually considered to indicate “interchangeability” between the tested method and the reference method.³⁶ The rationale for using 30% as suggested by Critchley, lies in the inherent precision of the tested method and the reference technique.³⁷ Critchley stated that if both methods tested have an inherent precision of 20%, the mean percentage error equals the square root of the product of the second power of the inherent precisions ie $\sqrt{(20^2 \cdot 20^2)}$ ie 28.4% which has been rounded off to 30%. However, since MPE is dependent on the inherent precision it is probably inappropriate to use 30% as the single cut off when approving a new method. Instead MPE should probably be analysed in the light of the inherent precisions of the tested method and the reference method.²⁶ Other studies suggests that one can even accept a mean percentage error of up to 45% if the monitoring device has apparent advantages.³⁸

Trending ability

The ability for a device to track changes are assessed by calculating the concordance (the proportion of measurements that change in the same direction when two techniques are compared). An exclusion zone of 15 % is generally used as to compensate for statistical noise.³⁵ The size of the exclusion zone is

related to the inherent precision of the reference methods and the LSC. The better the precision, the smaller the LSC and the smaller the exclusion zone should be considered.²⁶ Strictly speaking if a reference method for example has precision of 10%, the LSC is 14% ($10 \cdot \sqrt{2}=14$) and an exclusion zone of 15% should be considered.

Least significant change (LSC)

The LSC is the minimum change that can be measured by a device in order to recognize it as a real change and is defined by the following equation:

$$\text{LSC} = \text{inherent precision} \cdot \sqrt{2}.$$

If a reference method for instance has an inherent precision of 20 % one would need to design a protocol that changed the CO at least $20 \cdot \sqrt{2} = 28$ % for the changes to be trusted as real.²⁶ The concept of LSC is particularly important when evaluating trending ability and exclusion zone for a study as described above.

Choosing reference method

When validating a new monitoring technique, it should be tested against a reference method using the statistical principles described above. In the normal case, CO studies examining new CO methods are conducted by comparing a series of paired measurements between the tested method and a known reference, sometimes referred to as the “gold standard” method. These paired recordings are ideally done during both stable and unstable haemodynamic conditions²⁵ to test the limit of the method and to examine the capability of the device to track changes. The ideal reference method should have good inherent precision, good estimation of absolute values, excellent trending ability and a short response time.²⁵

However, one of the main problems when validating new monitoring methods and CO monitors in particular is the lack of a true gold standard. In fact, there is no absolute clinical gold standard for CO monitoring and most methods have inherent precisions around 10% at the best.³⁹ For many years, pulmonary artery thermodilution (PAC) have been considered a gold standard. However, even if performed under ideal circumstances, PAC generally holds a precision of only 20% to 10% at the best.^{40,41}

For laboratory use, ultrasonic flow probes placed around the pulmonary trunk (CO_{TS}) are generally regarded as gold standard. It has a reported inherent precision of less than 10%.⁴² For obvious reasons, CO_{TS} cannot be routinely used in humans. Instead methods such as PAC in adults or modified

CO₂ Fick and trans pulmonary thermodilution in children are regarded as gold standards. However, these methods generally have lower precision^{40,41} and need arterial and central venous access or even pulmonary artery catheterization. MRI is another method for CO estimation in children that is associated with good precision but has very limited role in intraoperative CO monitoring for evident reasons.⁴³

For FRC measurements, inhaled helium wash-in/wash-out has a reported precision of around 12%⁴⁴ during experimental conditions but the application of perioperative FRC monitoring devices are often subjected to off-label use and the reported precisions should probably be judged thereafter.⁴⁵

It is also important to consider if the tested method measures continuous or static values since validation of continuous methods may appear worse when compared to a reference methods that uses static values. This is particularly true for measurements during haemodynamic and respiratory instability where the continuous method could in fact be more accurate.⁴⁶

Summary of background

Even if a tested methods agreement with a "gold standard" is important, it is perhaps even more useful to evaluate if a new device can be used to initiate therapies and monitoring results of various interventions. To design a monitor that as close as possible produce values in agreement with that obtained by "gold standard" such as PAC may therefore be redundant. Thus, an important development in paediatric CO and FRC monitoring would be randomized outcome studies where the benefit of intraoperative CO/FRC monitoring to guide haemodynamic and ventilatory management is investigated against "best current practice".^{47,48,49,50,51,52}

FICKS PRINCIPLE AND DIFFERENTIATED FICK

The classic Fick method described by Adolf Fick 1870, is still considered a gold standard for CO measurement.⁵³ Fick's equation is derived from the conservation of mass during pulmonary gas exchange. CO, or rather non-shunted pulmonary blood flow, is calculated from the oxygen consumption and the difference in arteriovenous oxygen content according to the formula:

$$CO = \frac{VO_2}{(C_aO_2 - C_vO_2)}$$

Equation 1: VO₂ is oxygen consumption, CaO₂ pulmonary end capillary oxygen content and CvO₂ is mixed venous oxygen content.

The method requires calorimetry for VO_2 measurement, arterial and pulmonary artery access and is therefore less suitable for routine clinical monitoring.⁵⁴ The equation can however be modified to include CO_2 instead of oxygen. Replacing oxygen with CO_2 as a tracer and applying the same principles gives the following equation:

$$C = \frac{\text{VCO}_2}{(\text{C}_v\text{CO}_2 - \text{C}_a\text{CO}_2)}$$

Equation 2 VCO_2 is carbondioxide elimination, C_vCO_2 mixed venous carbondioxide content and C_aCO_2 is pulmonary end capillary carbondioxide content.

Using direct CO_2 Fick for CO estimation is applicable in clinical practice and the method has been validated in children.⁵⁵ However, in analogy with oxygen Fick, the method is resource demanding and therefore rarely used.

The concept of using CO_2 as a tracer results in a significant simplification in CO_2 -based CO-monitoring since all components of the equation system can be determined through non invasive measurements.⁵⁶ In 1988, Capek et al showed that C_aCO_2 , can be calculated from the alveolar carbon dioxide fraction using the CO_2 dissociation curve.⁵⁷ This concept allows for an easy way of estimating pulmonary end capillary CO_2 content but it doesn't take shunted CO_2 into account. Strictly speaking, all CO monitoring models based on CO_2 Fick can therefore only calculate non shunted pulmonary blood flow.⁵⁶

The other variable necessary for CO calculations, VCO_2 , can be determined using volumetric capnography.⁵⁸ Still, even if CO_2 and C_aCO_2 can be calculated from standard mainstream infrared CO_2 sensor measurements, complex and potentially harmful pulmonary artery access is still required.

The differentiated Fick method

The problem with the need for invasive C_vCO_2 measurement was partially overcome when Gedeon et al introduced the differentiated Fick method.⁵⁹ In differentiated Fick, simultaneous VCO_2 and C_aCO_2 estimations are done at two separate occasions differed by a small change in alveolar ventilation. The first paired recording is done in a period of steady state after which a sudden perturbation in CO_2 elimination is introduced and a second recording is done.⁵⁹

Provided that C_vCO_2 does not change during the brief change in CO_2 elimination, C_vCO_2 can be regarded as constant and thus shortened from equation 2. The subsequent equation for two time points with constant C_vCO_2 separated by a small change in CO_2 elimination can be expressed as;

$$Q = \frac{VCO_{21}}{(C_{v1}CO_2 - C_{a1}CO_2)} = \frac{VCO_{22}}{(C_{v2}CO_2 - C_{a2}CO_2)}$$

Equation 3: 1 and 2 indicates values at time point 1, steady state, and 2, after a change in CO₂ elimination.

From standard algebra the following can be derived;

$$X = \frac{A}{B} = \frac{C}{D} = \frac{(A-C)}{(B-D)}$$

Equation 3 can therefore expressed as;

$$Q = \frac{VCO_{21} - VCO_{22}}{(C_{v1}CO_2 - C_{a1}CO_2) - (C_{v2}CO_2 - C_{a2}CO_2)}$$

thus

$$Q = \frac{VCO_{21} - VCO_{22}}{(C_{v1}CO_2 - C_{v2}CO_2) - (C_{a1}CO_2 - C_{a2}CO_2)}$$

Given the presumption that C_vCO₂ is constant between the changes in VCO₂, C_{v1}CO₂ is equal to C_{v2}CO₂ therefore;

$$Q = \frac{VCO_{21} - VCO_{22}}{-(C_{a1}CO_2 - C_{a2}CO_2)}$$

$$Q = \frac{VCO_{21} - VCO_{22}}{C_{a2}CO_2 - C_{a1}CO_2}$$

$$Q = \frac{\Delta VCO_2}{\Delta C_a CO_2}$$

Provided that the partial pressure of end tidal CO₂ (PECO₂) is corrected for alveolar dead space, it can be assumed to equal C_aCO₂ which is in equilibrium with alveolar CO₂ partial pressure thus;

$$C_a CO_2 = S CO_2 \times PECO_2$$

Equation 4: S CO₂ is the solubility coefficient of CO₂ in blood and requires an estimate of the hemoglobin concentration and oxygen saturation SpO₂ obtained from pulse oximetry.

The change in end capillary CO₂ content (ΔC_aCO_2) between the two points of different CO₂ elimination is given by;

$$\Delta C_aCO_2 = S_{CO_2} \times (PECO_{21} - PECO_{22})$$

Equation 5

Substituting this in equation 1 gives us;

$$Q = \frac{\Delta VCO_2}{S_{CO_2} \times (PECO_{21} - PECO_{22})}$$

Equation 6

Equation 6 describes an elegant model for estimation of the pulmonary blood flow that participates in gas exchange, often called effective pulmonary blood flow (EPBF). By continuous monitoring of VCO₂ and PECO₂, combined with a solubility constant dependent on hemoglobin level, barometric pressure and oxygen saturation from pulse oximetry and mathematical processing, the model can be used for CO monitoring in virtually any mechanically ventilated patient without the need for complex vascular access.⁵⁶

The majority of the current CO₂ based CO monitoring devices are based on the principle described in Equation 6. The difference between the methods lies mainly in how the change in CO₂ elimination is created. The most commonly used approaches are cyclic variations in alveolar ventilation or partial rebreathing by variations in dead space.⁵⁶

Change in alveolar ventilation

In Gedeon's original study, alterations in respiratory rate was used to induce perturbations in CO₂ elimination. This was accompanied by a change in I:E ratio so that effectively only the end-expiratory pause was altered, providing the best possible conditions for pulmonary blood flow to remain constant throughout the change in ventilation. Measurements could be repeated at maximum every 15th minute, to ensure that any possible fluctuations C_vCO₂ caused by the respiratory pattern it self was minimized. The method showed good agreement against PAC when tested in anaesthetised dogs.⁵⁹

This method was further developed on 2002 with a modified mathematical formula and breathing pattern intended to cause less interference of the ventilation by shortening the perturbation periods from 15-30 seconds to a 3 s end inspiratory pause of a single breath, followed by 1-3 normal breaths to re establish steady state . Gedeon validated the breathing pattern against PAC in adult cardiac surgery patients with good agreement.¹⁹

Because of the large capacity for the lungs to store CO₂, any small change

in alveolar CO_2 concentration changes the equilibrium required for equation 6 to be valid. Even if $C_v\text{CO}_2$ inflow to the alveoli is constant during the breathing cycle, CO_2 can still be released to the alveoli from stores in the pulmonary tissue.⁶⁰ The CO_2 gas exchange process between blood and alveoli must therefore be separated from the effects of changes in CO_2 , stored in the lung tissue. Gedeon approached this problem by the adding another variable, effective lung volume (ELV) to the equation.⁶⁰ ELV can be viewed as a the combined volume of CO_2 participating in gas exchange and CO_2 stored in lung tissue. ELV should not be confused with the anatomical volume functional residual capacity (FRC) although several studies have indicated that they are strongly related.^{10,11,20}

Variations in dead space by partial rebreathing

Capek and Roy developed an alternative approach to induce perturbations in CO_2 elimination by cyclic variations in dead space. Dead space was varied by periods of partial rebreathing followed by normal ventilation. They found that even when the partial rebreathing period was limited to 30 s, $C_v\text{CO}_2$ remained largely constant allowing the method to give EPBF values every third minute thereby making it the first semicontinuous CO_2 based CO monitor. Capek and Roy integrated the hemoglobin level into the mathematical formula to account for the effect of the hemoglobin level on the CO_2 dissociation curve.⁵⁷ An important finding from this was that large changes in hemoglobin concentration could cause errors in the EPBF calculations making the system sensitive to shifts in hemoglobin.

A commercially available method, NICO (*NICO Respironics, Murrysville PA*) is available where also shunt fraction is accounted for using Nunns iso shunt fraction plots.⁶² In adult practice, several clinical studies comparing partial CO_2 rebreathing with PAC have shown a good level of agreement between the two methods.⁶³

Partial rebreathing by adding dead space have also been tested in children.⁶³ However the system is currently not validated for patients <15 kg or for tidal volumes <300 ml. It is technically challenging in children and requires a period of haemodynamic stability for CO measurement. This makes this type of partial rebreathing system less ideal for paediatric and neonatal use where airway patency and circulatory status may vary considerable during anaesthesia. Like other CO_2 -CO methods, the technique is also less reliable in situations with altered alveolar distribution of gas such as interstitial or obstructive lung disease.⁶⁵

Capnodynamics

In 2006, Peyton et al. described a continuous breath-by-breath capnodynamic approach to CO measurements based on differentiated Fick. Alveolar CO₂ changes were induced by serial changes in the tidal volumes in a preset rhythmic fashion.⁶⁶

The principle, closely related to partial rebreathing methods, is based on a molar balance for pulmonary gas exchange. The mathematical and physiological background for Peytons method is described below.

At any given time, the volume of CO₂ present in the lungs (VCO₂) can be described as;

$$VCO_2 = \frac{PACO_2}{PB} \times V_{effCO_2}$$

Equation 7. PACO₂ is alveolar partial pressure of CO, PB is ambient barometric pressure, and V_{effCO₂} is the effective lung volume for carbon dioxide (cf. effective lung volume, ELV from Gedeon et al 2002).

The rate of change in VCO₂, dVCO₂/dt, is dependent on influx of mixed venous CO₂, outflow of end capillary CO₂ and expired CO₂. This can be expressed as;

$$\frac{dVCO_2}{dt} = Q \times C_vCO_2 + VCO_2 - Q \times S_{CO_2} \times \frac{PACO_2}{PB}$$

Equation 8. VCO₂; rate of elimination of carbon dioxide for any given breath, C_vCO₂ is the fractional content of carbon dioxide in mixed venous blood. S_{CO₂}, the solubility coefficient of carbon dioxide in blood, for given conditions of temperature, hemoglobin, and oxygen saturation. PB barometric pressure.

Derivative of equation 7 gives

$$\frac{dVCO_2}{dt} = \frac{dPACO_2}{dt} \times \frac{V_{effCO_2}}{PB}$$

Equation 9

Substituting in equation 8 and transposing gives;

$$\frac{dPACO_2}{dt} \times \frac{V_{effCO_2}}{PB} - VCO_2 = Q \times C_vCO_2 - Q \times S_{CO_2} \times \frac{PACO_2}{PB}$$

Equation 10

Equation 10 contains three unknown variables, V_{effCO₂}, C_vCO₂ and Q. In steady state, the right side reflects total CO₂ production. The variables on the left hand side can be measured using volumetric capnography and analysis of end tidal partial pressure of CO₂.

Peyton used a continuous breath cycle consisting of six smaller and six

larger tidal volumes, creating a change in alveolar CO₂ continuously analyzed using the principle described in equation 10. The method was tested in mechanically ventilated sheep using transpulmonary ultrasonic flow probe as a comparator and showed good agreement of absolute values in periods of haemodynamic stability when compared to PAC.⁶⁶ In 2012, Peyton described the use of step reductions in respiratory rate from 14 to 6 breaths/minute for a period of 45 s accompanied by a change in I:E relationship from 1:2 to 1:6. This approach led to minimal interference with respiratory pattern and optimal conditions for stable mixed venous CO₂ content since it effectively only affects the duration of the end expiratory pause. The method showed good agreement against PAC and esophageal Doppler when tested in adult cardiac surgery and in liver transplant patients.⁶⁷

Peyton's solution was the first application of differentiated Fick for continuous breath-by-breath CO monitoring with a precision and accuracy well in level with other CO methods such as PAC.

Current capnodynamic equations

In 2013 Albu et al reported the use of a variant of the differentiated Fick equation, to estimate FRC in anaesthetized rabbits.¹¹ Changes in alveolar CO₂ were induced by variations in I:E relationship rather than manipulations of tidal volumes or respiratory rate. The continuous method used in Albus work is based on the molar balance for CO₂ and is closely related to eq 10;

$$ELV \cdot (F_A CO_2^n - F_A CO_2^{n-1}) = EPBF \cdot \Delta t^n \cdot (C_v CO_2 - C_c CO_2^n) - VT CO_2^n$$

Equation 11

ELV, effective lung volume (liter) containing CO₂ at the end of expiration; EPBF, effective pulmonary blood flow (liter min⁻¹); ⁿ, current breath; ⁿ⁻¹, previous breath; F_ACO₂, alveolar CO₂ fraction; C_vCO₂, venous carbon dioxide content (liters gas liter blood⁻¹); C_cCO₂ⁿ, lung capillary CO₂ content (calculated from F_ACO₂); VT CO₂ⁿ, volume (liter) of CO₂ eliminated by the current, nth, breath; Δ tⁿ, current breath cycle time (min).

By constantly changing the inspiratory and expiratory relationship in a preset rhythm of normal breaths with inspiratory/expiratory relationship of 1:2, followed by a number of breaths with an extended expiratory pause (approximately 2 seconds, causing a functional lower respiratory rate), alveolar carbon dioxide concentration changes approximately 0.5-1 kPa.⁶¹ This change in alveolar carbon dioxide concentration and elimination is related to the alveolar blood flow as described in Equation 11. Each breathing analysis sequence consists of a number of breaths, commonly 9 or 10.^{10,11,68,71} Every breath creates a new separate equation. Thus, a series of breaths generates an equal

number of equations with 3 unknown variables (EPBF, ELV, $C_v\text{CO}_2$). By optimizing the fit between the lung model and measured data, the equation system described above can be solved. The equation also makes it possible to quantify ELV. The calculations made are compared to an ideal one-lung compartment model. If the differences between measured data and data calculated from the ideal model are outside the limits of acceptance, no values of ELV or EPBF are obtained. This gives the method a built in error-detection system to avoid the risk of displaying false values.

Recent publications by Albu and Hällsjö Sander et al^{61,68,69} have described the use of this continuous dynamic capnography for assessment of CO and FRC in animals and by our research group also in infants.⁷⁰ Agreement with other CO established comparators has been encouraging in these studies and associated with good trending capabilities. The structure of the pattern has been refined and the method tested against “gold standards” under normal and variable physiological situations as described below.

Validation of capnodynamics for CO and FRC estimations

In Albus study from 2013, the capnodynamic approach described in equation 11 was used for ELV measurements in mechanically ventilated rabbits exposed to various PEEP levels. The method was validated against multiple breath helium wash out and a breathing pattern of five normal breaths followed by five breaths with approximately 2 seconds inspiratory pause.¹¹ ELV showed good agreement and ability to track changes in FRC in response to PEEP changes when compared with helium method. In another animal study by albu in 2015, ELV was compared against helium wash out and computer tomography (CT) at different PEEP levels before and after surfactant depletion in rabbits. Again, ELV showed promising capability of continuous lung volume monitoring before and after lung lavage.¹⁰

A study by Hällsjö et al tested a similar experimental respiratory pattern using six normal breaths and three breaths with 2 expiratory pause. The method was compared with SF6 tracer gas wash out in pigs exposed to a series of haemodynamic challenges.⁷¹ ELV showed a good agreement (mean percentage error 36%) and trending ability (concordance rate 93%) when compared with sulfur hexafluoride (SF6) wash out. A key finding in this study was that ELV measured with expiratory hold appeared robust to changes in CO and pulmonary blood flow. They concluded that an expiratory hold potentially influences pulmonary blood flow to a lesser extent than inspiratory hold thereby optimizing the conditions for constant $C_v\text{CO}_2$ during each measurement

cycle as stated by Gedeon.⁶⁰

In a porcine model with intact lungs, by Hällsjö et al, dynamic capnography was used according to eq 11, to compared DC with PAC and pulmonary artery flow probe in a study involving a series of haemodynamical challenges (68). A breathing pattern of five normal breaths and five breaths with inspiratory pause was used. Even if overall bias was low and trending abilities good, a mean percentage error was 48% indicating less than optimal agreement between the methods.

In a similar study 2015 using the same breathing pattern, dynamic capnography was tested in a porcine model of lung lavage-induced surfactant depletion. Lung lavage leads to impaired gas exchange and has been known to be an aggravating factor for other techniques based on differentiated fick methods.⁶⁹ When shunt fractions increased after lung lavage both the agreement and precision was impaired (mean percentage error increased from 36 to 70%), as with most described CO₂ based CO methods. However, trending ability was well-preserved even after the lavage.

In 2017, Sigmundsson et al examined the same breathing pattern as Hällsjö 2016⁷² to test the capnodynamic method by using a porcine ischemia-reperfusion and hypercapnia model to create rapid changes in C_vCO₂ levels. CO_{EPBF} accuracy was found to be impaired during periods of reperfusion but agreement was restored within 5 minutes after reperfusion. Prolonged hypercapnia did not affect CO_{EPBF} in this study.

Summary of dynamic capnography

The development of minimally invasive CO₂ based CO monitoring methods in the adult world has resulted in a number of breath-by-breath methods that are accurate and easy-to-use. The methods are currently tested in paediatric animal models and in the clinical context with promising results.

Current research has focused mainly on standardized validation against gold standards, mainly PAC and transpulmonary flow probe. The results are promising and dynamic capnography shows inherent precision and accuracy well in level with PAC for most situations. However, the real challenge for dynamic capnography, testing its clinical usefulness, remains to be carried out. Randomized trials of CO guided haemodynamic therapy are therefore most likely the next natural step in the process of determine whether dynamic capnography is an effective adjunct to the existing clinical monitoring, especially with respect to morbidity and mortality in critically ill children.

ASPECTS ON RESEARCH COLLABORATION WITH THE MEDICAL INDUSTRY

Paper I, II and III have all partially been funded by unrestricted grants from Maquet Critical Care AB, Solna Sweden, as stated in each manuscript. Research sponsored by the medical industry is occasionally met with doubt and even runs the risk of being seen as tainted. Rightly, this raises a few considerations worth highlighting.

Currently, non-government resources sponsor more than half of the investigation at Karolinska Institutet and the medical industry contributes significantly to this funding.⁷³ Firstly, it is important to emphasize that Karolinska Institutet encourages research collaboration with the medical industry, illustrated by a statement in the text “Corporate partnering at Karolinska Institute”⁷⁴:

“Karolinska Institutet welcomes and strongly encourages partnerships with industry in order to realize the potential of research findings and knowledge generated at the university. Partnerships are deemed important for building further excellence in research, education and innovation”

Furthermore, neither academia nor the medical industry is immune from ethical responsibility or liability and government funded projects offers no natural protection from factors that risk threatening the objectivity of research (please c.f. Macchiarini scandal).

With state-funded research constantly at risk of declining, academic institutions in general are increasingly relying on external funding. Following this intensified engagement between academia and industry, it is important to adapt an approach and awareness to protect the credibility of the partnership. As with any research project, regardless source of funding, accuracy and vigilance can minimize bias and provided that studies are planned, performed and reported to minimize this, by researches whose income is not depending on the method being evaluated, there is no decisive reason why the interests of the academia and industry can not be aligned in this context.

To further safeguard the academic integrity of this project, it was decided from the onset that the supervisors of the thesis was only academia-based.

3 AIMS

1. To assess the feasibility of CO_{EPBF} for agreement of absolute values and ability to track changes in CO when compared to the current standard, supra-sternal Doppler (CO_{SSD}), in the human clinical setting (Study I).
2. To validate CO_{EPBF} in a porcine model of selective pulmonary vasoconstriction, a situation challenging the EPBF method with regards to isolated right ventricular workload and intrapulmonary CO_2 content (Study II).
3. To characterize the PEEP-level needed to preserve FRC, ventilation homogeneity and allow for adequate CO_2 clearance in a paediatric model of laparoscopic surgery (Study III).
4. To compare capnodynamic ELV with the gold standard, helium wash-in/wash-out FRC method, at the PEEP level resulting in adequate lung homogeneity, in the same model of paediatric laparoscopic surgery (Study III).

4 METHODS

The methods used are described in more detail in each paper. Below is a general presentation of the methods used.

Settings and ethical permits

Study I is divided into two parts, one clinical and one experimental. The clinical part of study I was performed at the Karolinska University Hospital, Stockholm, Sweden and the experimental part of study I and II was performed in the Hedenstierna Laboratory, Uppsala University, Uppsala, Sweden. The clinical part of study I was approved by Internal Ethical Review Board approval and Parental Informed Consent (verbal and written) was collected before initiation of the study.

For the experimental part of study I and study II, approval from Uppsala Animal Ethics Committee Uppsala, Sweden was obtained.

Study III was performed at The Unit for Anaesthesiological Investigations, Dept. of Anesthesiology, Pharmacology and Intensive Care, University of Geneva, Geneva, Switzerland and the experimental protocol was approved by the institutional ethics committee for experimental research of the University of Geneva and animal welfare committee of the Canton of Geneva, Switzerland.

Further details of the ethical permits can be found in each paper.

Patients and animals

Patients (study I)

15 children (median age 8.5 months range 6-23 months; median weight 8.3 kg (range 7.8-10.5 kg), scheduled for surgical cleft lip-palate repair, were included. Exclusion criteria were concomitant systemic illness, known heart disease, ongoing medication, American Society of Anesthesiology risk class 3-4.

Anaesthetic procedure study I

Induction of anaesthesia was either inhalational (Sevoflurane) or intravenous (Propofol). Anaesthesia was maintained using a standardized intravenous regimen with propofol infusion mg kg^{-1} and bolus dose fentanyl 1 mcg kg^{-1} . Rocuronium 0.5 mg kg^{-1} was used for muscle relaxation. The patients were pre-oxygenated (FiO_2 : 1.0) and the airway was secured using a cuffed endotracheal

tube of age adequate dimension (Microcuff® Pediatric ETT, Kimberly Clark, Health Care, Atlanta, GA, USA) with the cuff inflated to allow an adequate airtight seal. Following endotracheal intubation the patient was connected to a ventilator equipped with additional software creating the breathing pattern required for CO_{EPBF} determination (Servo I, Maquet Critical Care, Solna, Sweden). This was subsequently followed by a standardized lung recruitment maneuver, using FiO₂ 0.3 with positive end expiratory pressure (PEEP) 8 cm H₂O and tidal volumes (TV) 6-8 ml kg⁻¹ for 2 min.

Research animals and preparation

Pigs (I, II)

In paper I 9 pigs with a mean weight of 23.6 kg (range 23.4 – 24.8 kg, age 6-8 weeks) were used. In paper II, 10 pigs with median weight 23.9 kg (range 23.5 – 24.6 kg, age 6 to 8 weeks) were used. Both study I and II used animals collected from the same breeding colony (Mångsbo Farm, Uppsala, Sweden).

Rabbits (III)

Eight adult rabbits (chinchilla breed), median weight of 3.7 kg (range 3.6-3.9 kg), were collected from the same breeding colony (University Farm Arare, Canton of Geneva, Switzerland).

Anaesthetic and surgical preparation study I and II

Shortly after arrival to the laboratory, the pigs were sedated with 0.04 mg kg⁻¹ atropine (NM Pharma AB, Sweden), 6 mg kg⁻¹ tiletamine-zolazepam (Zoletil, Vibrac Laboratories, France), and 2.2 mg kg⁻¹ xylazine chloride (Rompun, Bayer AG, Germany) administered intramuscularly. Infusion of 5 µg kg⁻¹ fentanyl (Fentanyl B. Braun, Germany), ketamine 30 mg kg⁻¹ h⁻¹, midazolam 0.1 mg kg⁻¹ h⁻¹ and fentanyl 4 µg kg⁻¹ h⁻¹ was used for maintenance of anaesthesia and rocuronium 2 mg kg⁻¹ h⁻¹ for muscle relaxation. Ringer's acetate 10 ml kg⁻¹ h⁻¹ was administered throughout the experiment.

After tracheostomy, normoventilation was achieved (pressure control mode with Servo I, Maquet, Solna, Sweden): tidal volume 10 ml kg⁻¹, FiO₂ 0.4 and PEEP 5 cm H₂O. A balloon-tipped 7.5 Fr PAC was inserted via the right jugular vein into the pulmonary artery and the jugular vein and carotid artery were cannulated for pressure recordings and blood sampling. A urinary Foley catheter was inserted into the urinary bladder. Through a left sided thoracotomy, an ultrasonic flow probe was placed around the pulmonary trunk for continuous measurement of CO_{TS} as shown in *Figure 4*. The thorax was then closed and the animals placed in a supine position. Lung

recruitment was done using a peak pressure of 20 cmH₂O, PEEP 10 cmH₂O with I:E 1:1, respiratory rate 10 for 2 minutes. The body temperature was maintained at 38 – 39C.

After preparation, the piglets were allowed a 15-minute stabilization period before the initiation of the study protocols.

Following the end of studies, the piglets were euthanized according to established standard at the laboratory.

Pressure readings and signals from the ultrasonic flow probe were sampled into a data acquisition system (version 3.2.7, Acknowledge, BioPac Systems, Santa Barbara, CA, USA).

Anaesthesia and surgical preparation III

The rabbits were anaesthetized by an intramuscular injection of xylazine (3 mg kg⁻¹) and ketamine (15 mg kg⁻¹) followed by an iv injection of propofol (2 mg kg⁻¹). Following tracheotomy, an endotracheal tube (4 mm i.d, Portex®, Smiths Medical, Kent, UK) was inserted into the distal trachea. The jugular vein and carotid artery were cannulated for pressure recordings and blood sampling. Mechanical ventilation was started (volume control mode) with tidal volume 7 ml kg⁻¹ and respiratory rate adjusted to achieve normocapnea (5.5-6.0 kPa) and using an inspired oxygen fraction (FiO₂) of 0.4. The ventilator was equipped with the same additional software as used in all studies, creating the breathing pattern required for ELV determination (Servo I, Maquet Critical Care, Solna, Sweden).

Anaesthetic maintenance was achieved by a continuous intravenous infusion of propofol (10 mg kg⁻¹ h⁻¹) and fentanyl (50 µg kg⁻¹ h⁻¹) via an ear vein. All rabbits received continuous Ringer-acetate infusion for volume compensation (4 ml⁻¹ kg⁻¹ h⁻¹). Atracurium was given for muscle paralysis (0.5-1.0 mg kg⁻¹ h⁻¹) after confirming an adequate level of anaesthesia and analgesia. Carbon dioxide was insufflated to the abdominal cavity through a small airtight incision, using a commercially available intra-abdominal CO₂ insufflation device for surgical use (Electronic Endoflator, Karl Storz, Tuttlingen, Germany) to regulate IAP. Airway pressure, heart rate and rectal temperature were displayed and stored on a computer at a sampling rate of 1 kHz via an analog/digital interface converter (ADInstruments, Powerlab model 8/35 and Lab-Chart 7, Dunedin, New Zealand). Following completion of the protocol, the animals were euthanized according to established standard at the laboratory.

Reference methods used for CO monitoring

Supra sternal pulsed Doppler (CO_{SSD}) (Study I)

CO_{SSD} was measured by a single highly experienced sonographer and the measurements were performed using a Philips ultrasound system (Philips CX 50, S8-3 probe Philips Healthcare 3000 Minuteman road Andover, MA 01810 USA).

After establishing a satisfactory quality 2D parasternal long axis view, the aortic diameter was measured at the aortic valve annulus. Two consecutive aortic diameter measurements were made and the mean of the two values was used for CO calculations. To minimize error in angulation, the ascending aorta was insonated from the jugular notch using 2D and color Doppler.⁷⁵ The flow velocity was measured with pulsed Doppler and the sample volume placed just above the aortic valve. Care was taken to find the maximum velocity and after a stable Doppler signal was obtained the velocity time integral (VTI) was recorded over 6-12 consecutive beats (mean 8) with no specific timing to any of the sections of the capnodynamic breathing cycle. The mean VTI for one beat was then multiplied by the aortic valve area and the heart rate to obtain CO. *Figure 3* below shows an example of a series of Doppler recordings made with CO_{SSD}.

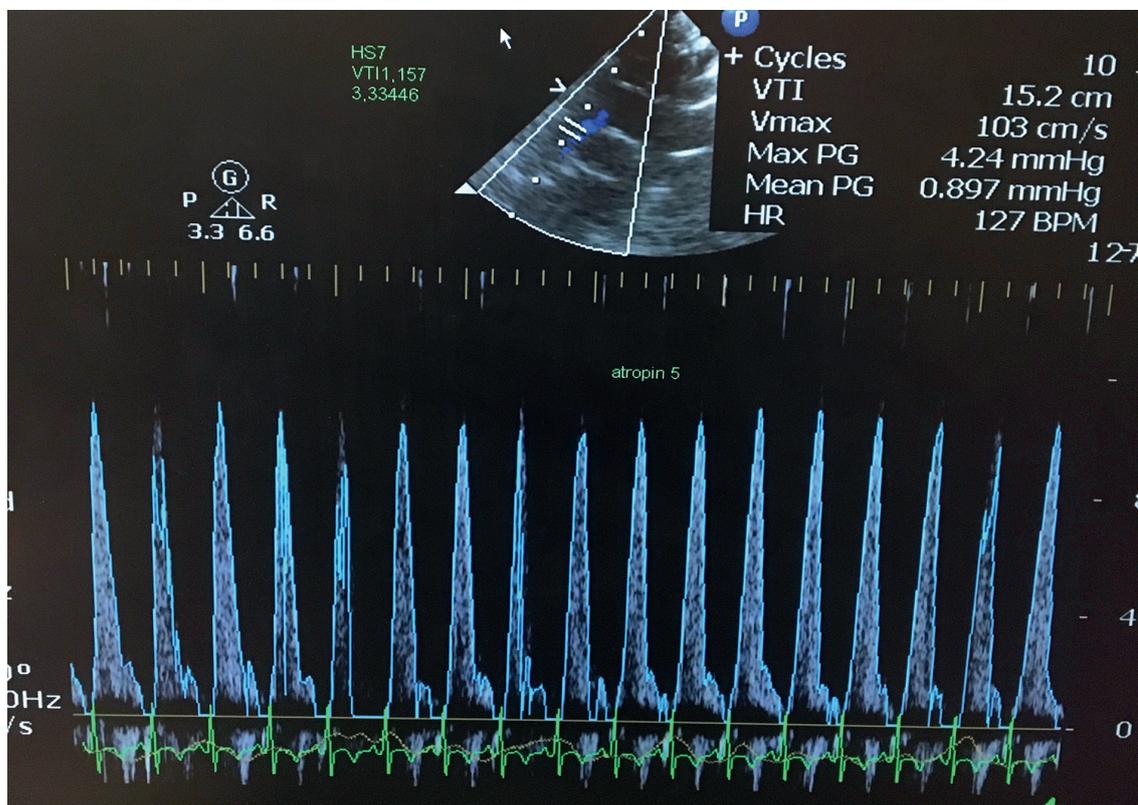


Figure 3. Doppler trace of the CO_{SSD} method. Each blue spike represents one VTI, the area corresponding to one stroke volume.

Pulmonary artery ultrasonic flow probe (CO_{TS}) (Study I and II)

Pulmonary artery ultrasonic flow probe (AU-series CO_Nconfidence Flowprobe® with ultrafit circle liner, Transonic System, Inc., Ithaca, NY, USA), CO_{TS}, was used in studies I and II. The signals from the ultrasonic flow probe were sampled into a sampling device (T 401; Transonic System, Inc., Ithaca, NY, USA) according to the instructions from the manufacturer. A filter of 0.1 Hz was used to display the average value over 10 seconds.

CO_{TS} estimates flow by passing ultrasonic signals back and forth through flowing blood, alternately intersecting the blood flow in upstream and downstream directions. A precise measure of the time it takes for the ultrasound wave to travel from one transducer to the other can then be used to measure flow (rather than to measure velocity, which is the base for Doppler estimations of flow).⁴² The CO_{TS} measurements is thus not depending on vessel cross sectional area. *Figure 4* below show the CO_{TS} probe in situ in a research animal.

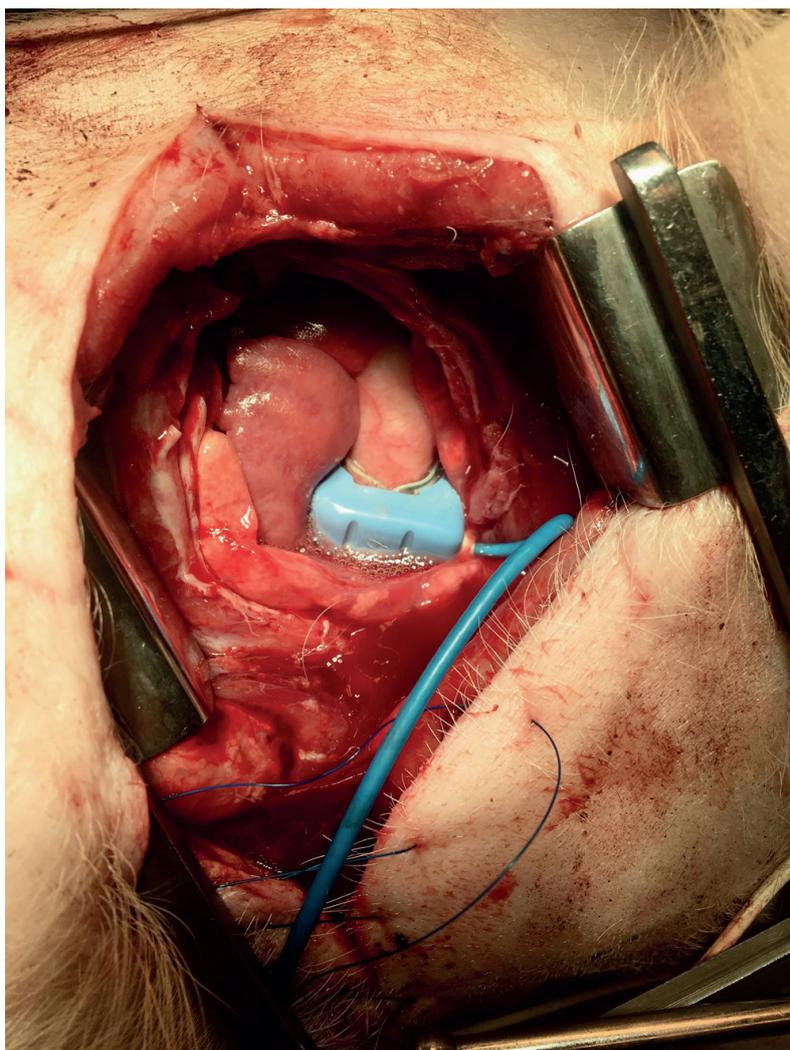


Figure 4.
The transpulmonary
flow probe in situ in a
research animal.

CO₂ based Cardiac Output estimation (CO_{Fick}) (II)

CO_{Fick} was determined by simultaneous arterial and mixed venous blood gas measurements and calculations of CO₂ content (ml dL⁻¹) in whole blood⁷⁶ and CO₂ elimination (VCO₂, ml min⁻¹) recorded by volumetric capnography via the ventilator (77). CO_{Fick} was determined using the equation below.

$$C = \frac{VCO_2}{(C_vCO_2 - C_aCO_2)}$$

VCO₂, CO₂ elimination (ml min⁻¹); cvCO₂, mixed venous CO₂ content (ml L⁻¹); c_aCO₂, arterial CO₂ content (ml L⁻¹).

VCO₂ measurement using volumetric capnography (I,II,III)

VCO₂ was calculated continuously with volumetric capnography using data from standard measurements of end-tidal CO₂ concentration with a mainstream infrared CO₂ sensor (Capnostat-3, Respironics Inc, Wallingford, CT, USA) inserted between the endotracheal tube connector and the standard Y-piece. Ventilation airflow was recorded by the standard Y-piece flow sensor of the Servo I ventilator. The tidal CO₂ elimination rate (VCO₂ ml min⁻¹) was then calculated using data from the ventilator and main stream capnograph with this setup.

End expiratory lung volume measurements with helium wash out

EELV in study III was determined by the helium multiple-breath wash-out technique (Exhalyzer D; ECO Medics AG, Dürnten, Switzerland). An ultrasonic flow meter (Spiroson Scientific; ECO Medics AG, Dürnten, Switzerland) was inserted between the endotracheal tube connector and the ventilator circuit to measure changes in molar mass of the respiratory gas. Helium was dispensed into the inspiratory limb of the ventilatory circuit to reach an end-inspiratory concentration of 4-5% after which the recording was started. The helium flow was then interrupted, and the washout dilution of the tracer gas was recorded. EELV was calculated from the changes in the He concentration during the wash out phase by a special software, (Spiroware, V1.4.3, ECO Medics AG, Dürnten, Switzerland).^{10,11}

Lung clearance index (LCI) (III)

LCI was calculated as the number of lung volume turnovers required to decreasing the helium concentration to 1/40th of the starting value.⁷⁸ LCI has been used in paediatric practice as an indicator of ventilation heterogeneity

and is a sensitive marker of small airway collapse.⁷⁸ The normal value in healthy children is approximately 6, with a low LCI value indicates a situation of more homogenous ventilation.⁷⁹

CO_{EPBF} and ELV estimations

In all three studies, CO_{EPBF} and ELV were calculated using the capnodynamic method. The development and theory behind the capnodynamic method, using the Differential Fick's principle, is described in further detail in the background section of this thesis. In short, the method is based a molar balance for CO₂ as described in the equation below:

$$ELV \cdot (F_A CO_2^n - F_A CO_2^{n-1}) = EPBF \cdot \Delta t^n \cdot (C_v CO_2 - C_c CO_2^n) - VT CO_2^n$$

ELV, effective lung volume (liter) containing CO₂ at the end of expiration; EPBF, effective pulmonary blood flow (liter min⁻¹); n, current breath; n-1, previous breath; F_ACO₂, alveolar CO₂ fraction; C_vCO₂, venous carbon dioxide content (litergas literblood⁻¹); C_cCO₂ⁿ, lung capillary CO₂ content (calculated from F_ACO₂); VT CO₂ⁿ, volume (liter) of CO₂ eliminated by the current, *n*th, breath; Δ tⁿ, current breath cycle time (min).

The ventilator continuously delivers a breathing pattern consisting of series of six breaths with an inspiratory/expiratory relationship of 1:2 followed by three breaths with approximately 2 seconds expiratory pause as shown in *Figure 5* below. The pause causes a 0.5 to 1 kPa increase in alveolar CO₂ concentration as well as an increase in CO₂ elimination, proportional to alveolar blood flow. The change in alveolar CO₂ concentration and elimination correlates to CO_{EPBF} and ELV as described in Equation 1. Each breathing analysis sequence consists of 9 breaths. Every breath creates one separate equation.



Figure 5. A snap shot of the ventilatory and capnographic trace from the experimental breathing pattern used in all studies ie a series of breaths with normal I:E relationship, followed by a series breaths with expiratory pause.

Thus, 9 breaths generate 9 equations with 3 unknown variables (ELV, EPBF and $C_v\text{CO}_2$). By optimizing the fit between an ideal one compartment lung model and measured data, the equation system described above can be solved.

Figure 6 below shows a snap shot of the computer system where EPBF and ELV values are displayed, along with the volumetric capnogram and other respiratory parameters.

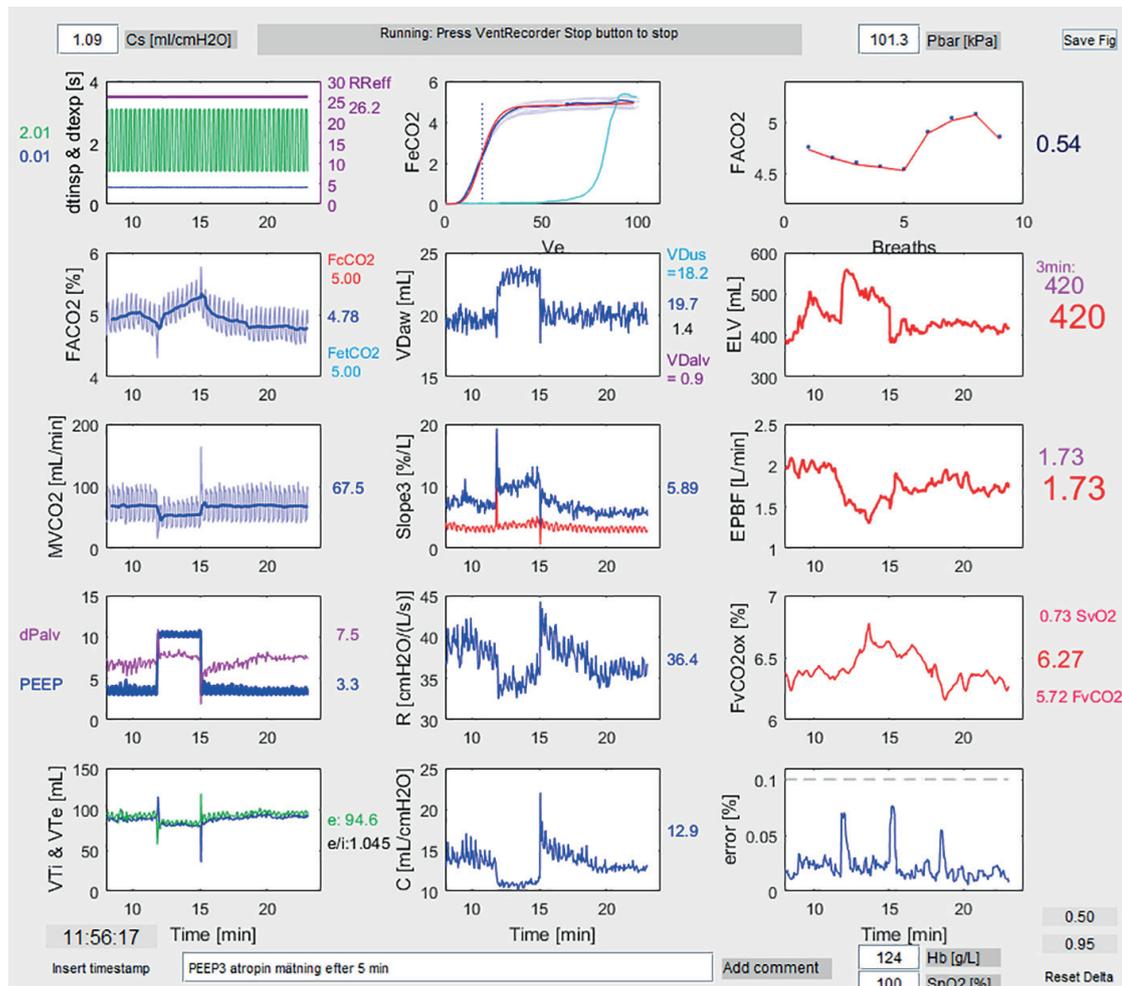


Figure 6. Snap shot of the experimental computer system used for data recording from study I. Note the EPBF and ELV panels to the far right and the PEEP panel to the left. EPBF decreases and ELV increases during elevation from PEEP 3 to 10 cmH_2O .

The method does not take shunted blood into account, but in the absence of cardiac or pulmonary shunting, CO_{EPBF} will be closely related to pulmonary blood flow and thus systemic CO .⁶⁰

Effective Lung Volume (ELV) (III)

The capnodynamic equation also makes it possible to quantify ELV (as shown in Equation 11), which corresponds to the lung volume participating in gas exchange including the airway volume up to the site of the CO_2 sensor and

the carbon dioxide dissolved in lung tissue and lung capillary blood.¹¹ Provided that adequate PEEP is applied, ELV is closely related to FRC during most circumstances.^{10,11,71}

Mainstream capnography (I, II and III)

CO_{EPBF} as well as ELV was calculated continuously using data from standard measurements of end-tidal CO₂ concentration with a mainstream infrared CO₂ sensor (Capnostat-3, Respironics Inc, Wallingford, CT, USA) inserted between the endotracheal tube connector and the ultrasonic flow meter. Ventilation airflow was recorded by the standard Y-piece flow sensor of the Servo I ventilator. The tidal CO₂ elimination rate (VCO₂ ml min⁻¹) was calculated using data from the ventilator and mainstream capnograph.⁵⁵

All lung volumes are corrected to Body Temperature and Pressure Saturated (BTPS).

The mathematical analysis required for the continuous CO_{EPBF} and ELV calculation in I, II and III was done in Matlab (MatlabTM, The Mathworks Inc, Natick, MA, USA).

Protocols

Inherent precision measurements I, II, III

Inherent precision measurements for DC were performed in all studies during stable baseline conditions, before the starting the study protocols. In study I clinical part, inherent precision was calculated from three initial paired CO_{EPBF} and CO_{SSD} baseline recordings. In the laboratory part of study I and in study II, 10 consecutively paired recordings 1 minute apart of CO_{EPBF} and CO_{TS} were used.

In study III, inherent precision of ELV was determined for each PEEP level using continuous measurements recorded over 3 minutes (about one hundred ELV values).

Study I

In study 1 CO_{EPBF} was validated against CO_{SSD} during clinical conditions. Due to the variable performance of CO_{SSD} , a porcine study was subsequently performed that mimicked the clinical protocol but instead we used CO_{TS} as comparator. *Figure 7* below shows the scheme of the protocol for study I clinical part.

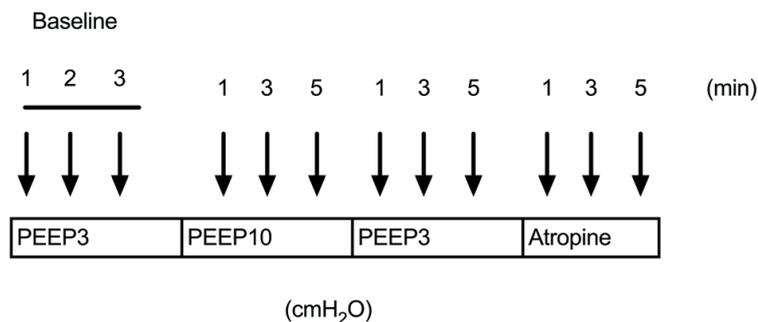


Figure 7. Scheme of the study protocol for the clinical part of Study I.

After a five-minute stabilization period following endotracheal intubation the study sequence was performed.

- Three baseline CO measurements were performed one minute apart, CO_{SSD} and CO_{EPBF} recorded simultaneously.
- PEEP was increased from 3 to 10 cmH₂O (expected to produce a reduction of CO). CO measurements were performed at 1, 3 and 5 minutes following the PEEP increase.
- PEEP was then turned down to 3 cmH₂O and new baseline recordings of CO were performed after 1 and 3 minutes.
- Finally the patients were administered an intravenous bolus of Atropine 20µg kg⁻¹ (expected to increase CO). CO_{EPBF} and CO_{SSD} were then recorded 1, 3 and 5 minutes after atropine administration, following which the protocol was stopped.

Protocol experimental part

The pigs were subjected to the same study protocol as used in the clinical part of the study with the following exception:

- Based on previous experience, piglets need $40 \mu\text{g kg}^{-1}$ of intravenous atropine to display a reliable haemodynamic response and was, thus, used instead of the $20 \mu\text{g kg}^{-1}$ used in the clinical part of the study.

Study II

In study II we compared CO_{EPBF} with CO_{Fick} and CO_{TS} for its ability to estimate absolute values and track changes in CO in a porcine model of hypoxia-induced selective pulmonary hypertension and subsequent inhaled nitric oxide treatment.

After baseline recordings, FiO_2 was decreased in two steps from 1.0 to 0.5 and 0.21. At FiO_2 below 0.21, inspired oxygen concentration was then slowly reduced in small increments, approximately every tenth minute using a gas mixture of air and nitrogen to achieve a 100% increase in PVR from the baseline recorded at FiO_2 1.0. 20 PPM inhaled nitric oxide was then added by an inhaled nitric oxide supplying device (SoKinox™, Air Liquide, Paris, France). Three minutes after the inhalational NO concentration reached 20 PPM, paired recordings of all CO_{EPBF} , CO_{Fick} and CO_{TS} were done. FiO_2 was then gradually increased in small increments to 0.21, and then stepwise to 0.5 and 1.0. At each step, time was allowed for haemodynamic stabilization before simultaneous CO_{EPBF} , CO_{TS} and CO_{Fick} recordings were done. *Figure 8* shows the study protocol for study II.

1.0	0.5	0.21	0.16	0.16+iNO 20	0.21+iNO 20	1.0+iNO 20
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Figure 8. Scheme of the study protocol for Study II. CO_{EPBF} , CO_{TS} and CO_{Fick} was recorded simultaneously at all seven time points illustrated.

Study III

In study III we examined the optimal PEEP level needed to preserve FRC, LCI, and VCO_2 during a model paediatric of laparoscopy with CO_2 pneumoperitoneum. We also aimed at validating ELV for its capability of bedside FRC estimations in the same setting.

Before the start of the protocol, the lungs were recruited using PEEP 3 cmH_2O , 14 ml kg^{-1} tidal volume while applying a 10 seconds inspiratory hold. This procedure was repeated once and used as standardized recruitment between PEEP level changes throughout the protocol.¹⁰ After this, the rabbits were ventilated in the volume control mode, tidal volumes of 7 ml kg^{-1} , PEEP

3 cmH₂O and FiO₂ 0.4. Respiratory rate was adjusted to reach normocapnea (5.5-6 kPa). The capnodynamic breathing pattern used for ELV estimations was then started.

Paired ELV and EELV measurements were made at IAP 0 mmHg and PEEP of 3 cmH₂O. The IAP was then randomly varied between 0, 6 and 12 mmHg. The paired recordings were collected 3 minutes after the desired IAP level had been achieved. This was repeated after PEEP had been increased from 3 to 6 and 9 cmH₂O, respectively. For EELV and LCI calculations, two separate helium wash outs were made after recording the ELV, and the average was used for further calculations. ELV is reported as an average of ELV values from 60 seconds preceding the helium wash out (about 30 ELV values). Blood gases were drawn immediately before the helium wash outs to determine paCO₂. Mean arterial blood pressure (MAP) was also recorded for each step. *Figure 9* below shows the scheme used in study III.

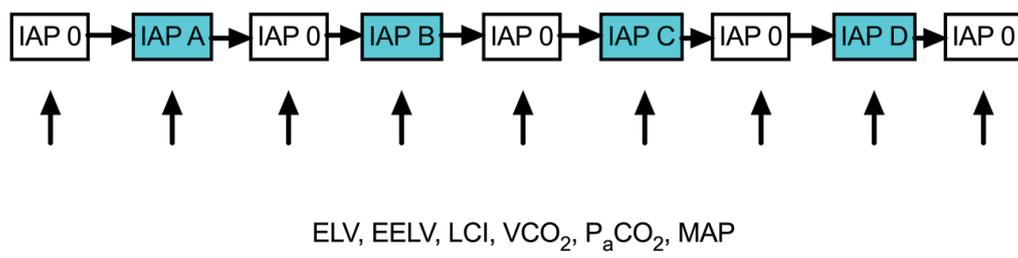


Figure 9. Scheme of the study protocol for Study III. IAP A, B, C and D, where IAP was randomly varied between either A/B=6 mmHg and C/D=12 mmHg or A/B=12 mmHg and C/D=6 mmHg.

Statistical methods

The statistical methods used are presented in each paper and the principles behind them are discussed in further detail in the background section of this thesis.

Distribution of data was checked with D’Agostino and Pearson omnibus K2 test for all studies. In study III a one-way ANOVA for repeated measurements and Tukey’s multiple comparison test was used to assess variations in respiratory and haemodynamic parameters for each PEEP level. A paired t-test was used to detect significant differences between ELV and EELV at individual IAP steps within a PEEP level. P<0.05 was considered to indicate statistical significance.

Precision

Inherent precision was defined as two times the coefficients of variation (CV=SD/mean).²⁶

Agreement and correlation between Dynamic Capnography and reference method

Agreement and limits of agreement (bias +/- (1.96xSD)) between CO_{EPBF}/ELV and reference methods were done with Bland Altman analysis in all studies.²⁸ Bias is expressed as the mean difference between CO_{EPBF} and the reference methods.

Correction for repeated measurements was not done given that each measurement is performed in a new situation where the reference value varies in a minor to moderate manner and thus represents independent serial measurements rather than repeated measurements.^{30,31}

Mean percentage error for CO_{EPBF} and the reference methods were defined as 1.96 x SD of the difference between the techniques divided by the mean of the reference technique as suggested by Critchley.³⁶ A priori, a mean percentage error less or equal to 30% was considered to indicate clinical useful reliability of CO_{EPBF} compared with the reference method.³⁶

In study III, correlation analyses between ELV and Helium wash-out were done using Pearson's correlation coefficient test.

Trending – ability to detect change

The ability of CO_{EPBF} and ELV to track changes in CO and FRC, was assessed by concordance calculation (the proportion of measurements that change in the same direction when CO_{EPBF} or ELV and reference method are compared). In study I and II, a 10% central exclusion zone was chosen, extrapolated from the precision of the reference methods. For CO_{Fick} vs CO_{EPBF} in study II and for Helium wash-out vs ELV in study III, a standard 15% zone was used.³⁷

Statistical Software

GraphPad Prism (version 7.0 for Windows, GraphPad Software, San Diego, CA, USA) was used for all statistical calculations and Microsoft Excel for Mac 2011 version 14.5.7 for data handling.

5 RESULTS

All animals in the experimental parts survived the experiment. Parts of data from one animal was excluded from paper II and III due to technical issues with data sampling.

STUDY I

The calculated inherent precision was $\pm 4\%$ and $\pm 12\%$ for CO_{EPBF} and CO_{SSD} , respectively in the clinical part and $\pm 6\%$ and $\pm 4\%$ for CO_{EPBF} and CO_{TS} , respectively, in the laboratory part.

In both the clinical and experimental sections, the haemodynamic interventions lead to relatively small changes in the range of 10% in CO (*Figure 10*).

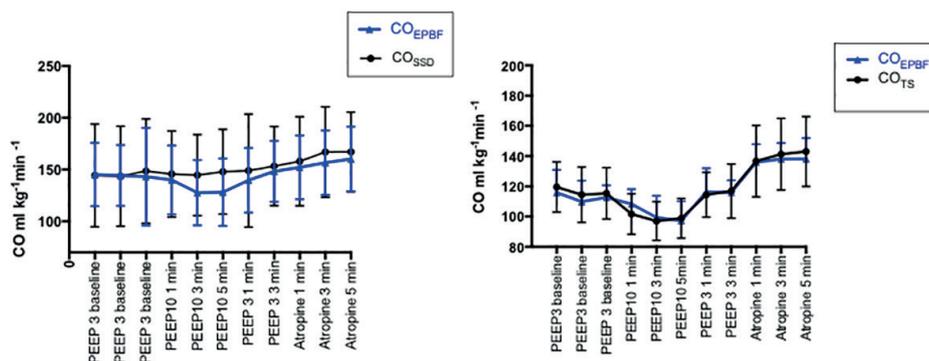


Figure 10. Time plot showing the relation between CO_{EPBF} and CO_{SSD} to the left and between CO_{EPBF} and CO_{TS} to the right during the various haemodynamic challenges. Values are mean \pm SD. $n=15$ in clinical study, $n=9$ in experimental study. Note that CO in the human study is age dependent causing a large spread (cf. the reduced spread in the animal study).

Agreement

Agreement for all paired data in the clinical section showed a mean bias between CO_{SSD} and CO_{EPBF} of $8.1 \text{ ml kg}^{-1} \text{ min}^{-1}$, 95% limits of agreement of -82 to $+66 \text{ ml kg}^{-1} \text{ min}^{-1}$, and a mean percentage error of 48%.

In the experimental part, agreement of mean bias between CO_{EPBF} and CO_{TS} was $18 \text{ ml kg}^{-1} \text{ min}^{-1}$, with 95% limits agreement of -36 to $+38 \text{ ml kg}^{-1} \text{ min}^{-1}$, and a mean percentage error of 31%.

Trending ability

Concordance rate between CO_{EPBF} and CO_{SSD} was 64% and 95% between CO_{EPBF} and CO_{TS} .

STUDY II

The calculated inherent precision were $\pm 6\%$ for CO_{EPBF} , $\pm 4\%$ for CO_{TS} and $\pm 28\%$ for CO_{Fick} .

The hypoxic challenge and subsequent inhaled nitric oxide treatment resulted in moderate haemodynamic changes in all animals as shown in *Figure 11*.

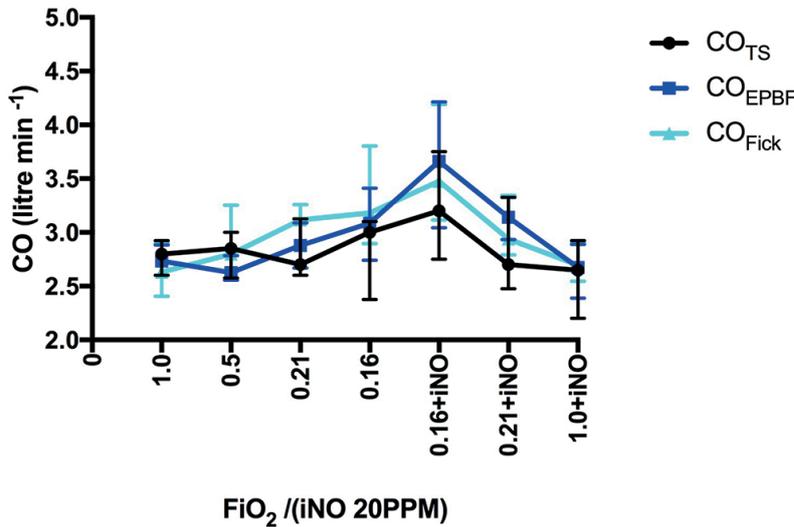


Figure 11. Time plot showing the relation between CO_{EPBF} and CO_{TS} and CO_{Fick} during the various states of hypoxic pulmonary hypertension. N=8-10. Values are median (range).

Median CO at baseline FiO_2 1.0 was 2.7 L min^{-1} (2.6 to 2.9) for CO_{EPBF} , 2.8 L min^{-1} (2.6 to 2.9) for CO_{TS} and 2.6 L min^{-1} (2.4 to 2.9) for CO_{Fick} . Overall, CO_{EPBF} displayed parallel changes when compared to the CO_{TS} and CO_{Fick} . Adding inhaled nitric oxide resulted in an increase in CO for all methods. FiO_2 concentration of 0.21 and above caused a decrease and return to baseline CO for all three methods as FiO_2 reached 1.0.

Agreement

For CO_{EPBF} vs CO_{TS} , agreement for all paired data showed a mean bias between CO_{EPBF} and CO_{TS} of 0.2 L min^{-1} (95% limits agreement of -0.5 to $+0.9 \text{ L min}^{-1}$) and a mean percentage error of 25%. Agreement between CO_{EPBF} and CO_{Fick} for all paired data showed a mean bias of -0.1 L min^{-1} (95% limits agreement of -0.9 to $+0.6 \text{ L min}^{-1}$) with an associated mean percentage error of 25%.

The corresponding values for CO_{TS} vs CO_{Fick} was a mean bias of -0.1 L min^{-1} (95% limits agreement of -0.6 to $+1 \text{ L min}^{-1}$), with an associated mean percentage error of 30%.

Trending ability

The concordance between CO_{EPBF}/CO_{TS} and CO_{EPBF}/CO_{Fick} was 86% and 81% respectively.

STUDY III

The inherent precision for ELV was 19%, 16% and 14% for PEEP 3, 6 and 9 cmH₂O respectively at IAP 0 mmHg. Inherent precision for helium wash out has previously been shown to be approximately 12 %.⁴⁴

Changes in respiratory parameters

p_aCO₂ and VCO₂

Arterial *p_aCO₂* did not show any significant changes when increasing IAP from 0 to 6 to 12 mmHg at PEEP 9 cmH₂O whereas a significant increase was seen for both PEEP 3 and 6 cmH₂O when IAP was increased from 0 to 6 (p=0.03 and 0.005 respectively). Increase of IAP from 6 to 12 mmHg did not cause any further significant changes in *p_aCO₂* at any PEEP level (Figure 11). *VCO₂* did not show any significant differences between IAP 0, 6 and 12 mmHg at PEEP level of 3 cmH₂O. When increasing IAP to 6 mmHg however, a significant increase in *VCO₂* was seen at PEEP 6 and 9 cmH₂O (p<0.0001 and p=0.0004 respectively). Increase of IAP from 6 to 12 mmHg did not cause any further changes in *VCO₂* at any PEEP level (Figure 12).

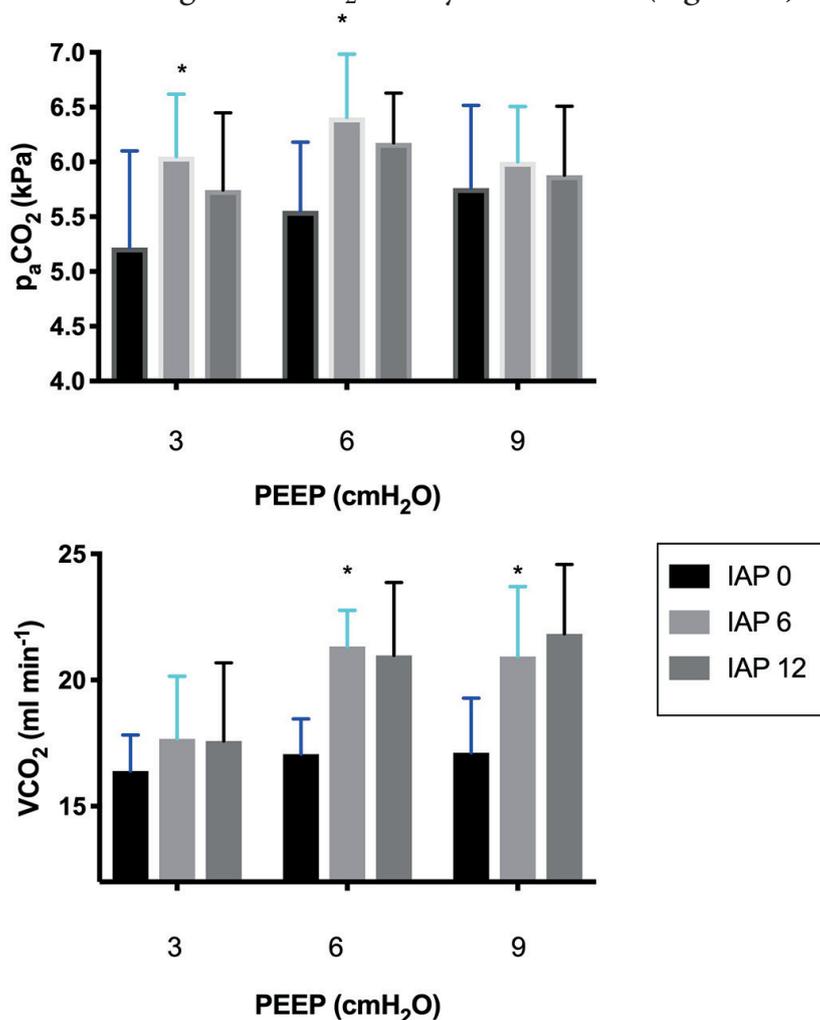


Figure 12. Changes in *p_aCO₂* (kPa) in arterial blood and *VCO₂* (ml min⁻¹) in response to increased PEEP for each IAP level. Values are mean±SD. n=7-8. *: P<0.05 IAP 0 mmHg vs IAP 6 mmHg within a PEEP.

Changes in Lung clearance index

PEEP 9 cmH₂O was the only PEEP level capable of preserving LCI at a level close to normal values for all IAP levels. Furthermore, LCI was significantly lower at PEEP 9 cmH₂O for all IAP levels when compared to PEEP 3 and 6 cmH₂O ($p < 0.05$).

Changes in EELV and ELV

As expected, going from PEEP 3 to 6 and 9 cmH₂O resulted in a significant increase in both EELV and ELV at IAP 0 mmHg ($p < 0.0001$). EELV displayed a significant decrease from IAP 0 to 6 mmHg for all PEEP-levels ($p < 0.0001$). At PEEP 9 cmH₂O, a significant decrease in EELV between IAP 6 and 12 mmHg was also noted, ($p < 0.0001$).

At PEEP 3 cmH₂O, ELV decreased significantly from IAP 0 to 6 mmHg ($p = 0.04$). No significant changes in ELV were detected for PEEP 6 cmH₂O whereas ELV decreased significantly between IAP 0 and 6 when measured at PEEP 9 cmH₂O ($p < 0.0001$).

At IAP 0 mmHg, ELV and EELV showed a strong and significant correlation for all PEEP levels ($r = 0.78$, $p < 0.0001$). No significant correlation between ELV and EELV were found for PEEP 3 and 6 cmH₂O when comparing all IAP levels. However, at PEEP 9, ELV and EELV showed a strong and highly significant correlation for all IAP levels ($r = 0.73$, $p < 0.0001$). *Figure 13* below shows the relation between ELV and EELV for all IAP levels at 9 cmH₂O.

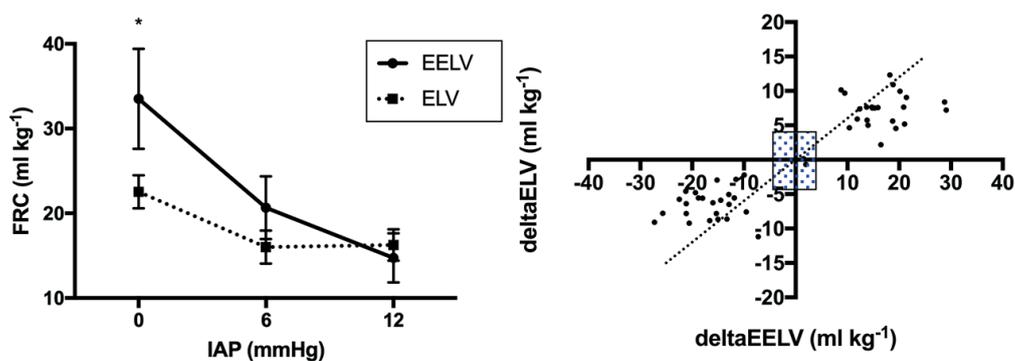


Figure 13. Left: Changes in ELV and EELV at PEEP 9 cmH₂O. Values are mean \pm SD. *: $P < 0.05$ ELV vs. EELV within an IAP. $n = 7$. Right: Four-quadrant plot of the concordance between ELV and EELV at PEEP 9 cmH₂O when IAP varies between 0, 6 and 12 mmHg. 46 paired delta values. The central boxed area represents a 15 % exclusion zone (4 ml kg⁻¹). The concordance rate is 100%. Dotted line represents line of identity. $n = 7$.

Agreement ELV and EELV

Bland-Altman analysis were only performed for recordings made at PEEP 9 cmH₂O since this was the only PEEP level showing significant correlation between ELV and EELV. Agreement for all paired data at PEEP 9 cmH₂O were

-4 ml kg⁻¹, 95% limits of agreement -18 to 9 ml kg⁻¹. For PEEP 9 cmH₂O, ELV values were found to be significantly lower than EELV at IAP 0 mmHg (p=0.002) whereas no significant differences were found between ELV and EELV at IAP 6 (p=0.06) and IAP12 mmHg (p =0.7).

Trending ability

The concordance rate between EELV and ELV was calculated for PEEP 9 cm-H₂O only (this PEEP level being the only level displaying significant a correlation between ELV and EELV). Using a 15 % exclusion zone, concordance was 100 %.

Changes in MAP

MAP increased significantly when going from IAP 0 to 6 mmHg at PEEP 6 and 9 cm H₂O (p=0.006 and p=0.03 respectively). No statistically significant differences in MAP were seen between the different PEEP levels within each IAP level (p<0.05).

6 DISCUSSION

Based on an initial series of promising animal experiments covering the use of the capnodynamic concept in estimating effective pulmonary blood flow,^{10,11,68,69,71,72} we sought to examine CO_{EPBF} as well as ELV for its performance in the clinical paediatric population and in paediatric experimental animal settings. We did this using study designs and statistical methods as recommended by the current consensus in the CO research community. In study I and II, we found an overall good agreement for absolute values between dynamic capnography and reference methods based on transpulmonary ultrasound and invasive CO_2 -Fick. In the clinical setting dynamic capnography appeared to perform in a more expected manner as compared to the reference method CO_{SSD} . In study III we found that dynamic capnography adequately reflects Helium wash-in/wash-out end expiratory lung volumes, provided that adequate PEEP is maintained. We also showed that this level of adequate PEEP, guarantees sufficient CO_2 removal and preservation of lung homogeneity under laparoscopic conditions. The results and implications of these findings are discussed in further detail below.

CO_{EPBF} in the clinical setting

The main findings in study I was that CO_{EPBF} appears to be a reliable technique to monitor even minor CO deviations in children during anaesthesia. In the laboratory part of study I, CO_{EPBF} generated absolute values very close to the gold standard reference CO_{TS} .

Aspects on the reference method CO_{EPBF}

The calculated inherent precisions of CO_{EPBF} and CO_{SSD} were found to be within acceptable limits. CO_{SSD} did not detect the expected reduction in CO in response to an increase in PEEP whereas this reduction was detected by CO_{EPBF} . There are several reasons for this, but may partly be due to the complexity and more situation-dependent nature of the CO_{SSD} method. Furthermore, the maximum decrease in CO in response to PEEP was in the region of 15%, making it difficult for CO_{SSD} to detect this given that its measured inherent precision of 12% requires a least significant change of $12\sqrt{2}$ ie approximately 17%. The fact that the elevation in CO in response to atropin was

detected by CO_{SSD} is more easily explained since this is related to the increased heart rate, conditions easily detected by CO_{SSD} .

The accuracy of CO determination using CO_{SSD} is dependent on a precise estimation of the diameter of the aortic outflow tract since this factor is included in the equation by the power of two (area of the aortic outflow tract = $\pi \times d^2/4$). In study I, the mean value of two different diameter measurements were used for CO_{SSD} calculations and this has obvious implications for the accuracy of the calculated values. However, this cannot explain why CO_{SSD} failed to detect the predicted reduction of CO caused by increased PEEP.

Increasing PEEP from 3 to 10 cmH₂O caused a greater degree of lung expansion, thus altering intrathoracic conditions, making it more challenging to obtain adequate ultrasound projections. These alterations in intrathoracic conditions required the sonographer to make continuous small adjustments with the ultrasound probe thus potentially affecting the inherent precision of the reference method. Therefore it cannot be excluded that despite using an experienced sonographer, this may have added an imprecision caused by variations in the angle of the ultrasound beam to the measured blood flow, a known weakness of the CO_{SSD} technique ^{2,80}

However, at PEEP 3 cmH₂O the geometry of the thorax is constant between recordings and minimal or no ultrasound probe adjustments were needed. During these circumstances, CO_{SSD} could easily detect the CO increase caused by atropine. CO_{EPBF} on the other hand, did not appear to be affected by any PEEP induced changes in the geometry of the chest cavity.

Since CO_{SSD} , unlike CO_{EPBF} , responded to the PEEP increase in an unexpected manner in the clinical study, we decided to use the exact same research protocol in an animal laboratory setting. This allowed us to compare CO_{EPBF} with the high precision gold standard CO_{TS} , to assess the agreement between the two methods and to investigate how pulmonary blood flow is affected by the haemodynamic interventions of the clinical protocol.

CO_{EPBF} in the laboratory setting of the clinical protocol

The calculated inherent precision of CO_{EPBF} was of the same order of magnitude as in the clinical trial and compared well with the calculated inherent precision of CO_{TS} .

As in the clinical protocol, CO_{EPBF} detected a decrease in CO in response to elevations in PEEP. The same decrease was also detected by CO_{TS} , supporting the view that CO decreased in response to PEEP elevations also in the clinical part even if CO_{SSD} failed to detect this (for reasons discussed above).

Overall, CO_{EPBF} and CO_{TS} showed good agreement of absolute values for all paired recordings throughout the study protocol with a mean percentage error of 31%, ie very close to the a priori set 30% limit for maximum acceptable mean percentage error.³⁶ From a practical perspective we therefore advocated that the values obtained with CO_{TS} and CO_{EPBF} can be regarded as near enough equal when measured under these circumstances.

Trending ability in the clinical and experimental context

The concordance rate between CO_{EPBF} and CO_{TS} was 95 % in the experimental study and 64 % in the clinical study. There are a number of potential explanations for the relatively poor concordance rate in the clinical study. For obvious reasons, in our clinical study, the magnitude of the acceptable haemodynamic provocations were limited by ethical restrains. We were therefore forced to induce relatively small CO variations. As mentioned above, the calculated inherent precision for CO_{SSD} made it difficult for this reference method to detect these comparatively small changes in CO, making proper determinations of trending ability challenging.

In previous CO_{EPBF} studies using animals, the concordance between CO_{EPBF} and reference methods have generally been very high, even up to 100% in some studies.⁷¹ However, in these studies, CO alterations have been large, usually well above the methods inherent least significant change limits, thus resulting in a higher probability of good concordance.

Contrary to the clinical study, concordance rate was found to be very good (95%) in the experimental study. The protocol in the experimental animal part, induced haemodynamic changes of the same magnitude as in the clinical protocol. In the experimental study however, both CO_{EPBF} and CO_{TS} detected these changes with a high concordance rate, thus suggesting that the lower concordance rate in the clinical part of the study possibly could be attributed to the less reliable performance of CO_{SSD} .

The main finding in study II was that CO_{EPBF} was able to estimate and trend CO values with excellent correlation to the reference methods CO_{Fick} and CO_{TS} during hypoxic selective pulmonary vasoconstriction and subsequent inhaled nitric oxide treatment.

Hypoxic challenge of the capnodynamic method

Selective hypoxic pulmonary vasoconstriction with subsequent pulmonary hypertension challenges a number of important factors with regards to the underlying assumptions of capnodynamic method, making this condition particularly interesting to examine. Selective hypoxic pulmonary vasocon-

striction will primarily result in increased workload for the right ventricle ie potentially affecting pulmonary blood flow. Furthermore, pulmonary vasoconstriction will recruit blood from small vessels within the lung tissue, thereby potentially altering one of the prerequisites of the capnodynamic method, namely a stable inherent CO₂ content of the pulmonary tissue including small lung vessels¹. There is therefore a risk that the inherent CO₂ content in the lung decreases as a consequence of pulmonary vasoconstriction, potentially affecting the accuracy of the capnodynamic method.

The capnodynamic principle is based on estimations of lung end capillary CO₂ content, calculated from measurements of end tidal CO₂ concentration. The physiological and mathematical principle behind this is discussed in further detail in the background section of this thesis. Hypoxia affects the CO₂ content in whole blood by increasing CO₂ loading and shifting the CO₂ dissociation curve upwards via the reverse Haldane effect⁸¹ as shown in *Figure 14*. At the steep part of the hemoglobin oxygen dissociation curve, oxygen saturation can change quite dramatically in response to a given change in pO₂.⁸² In fact, pO₂ has been shown to oscillate during normal mechanical ventilation in healthy lungs⁸³ and these oscillations are probably even more pronounced during hypoxia due to the steep slope of the hemoglobin oxygen dissociation curve. Added to this, the experimental breathing pattern we used, could potentially contribute to make oscillations even more significant since this pattern increases variations in alveolar O₂ and CO₂ compared to a normal breathing cycle.

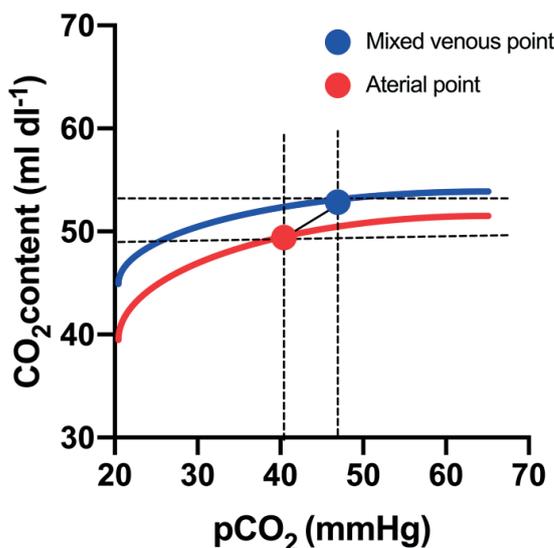


Figure 14. CO₂-dissociation curve and the Haldane effect modified according to Teboul et al.⁸¹

Under hypoxic conditions in our experimental setup, the position of the CO₂ dissociation curve could therefore vary slightly, thus theoretically affecting the absolute values of the calculated CO_{EPBF}.

However, the issues addressed above did not seem to affect the CO monitoring ability of CO_{EPBF} compared to CO_{Fick} and CO_{TS} in any relevant manner. Hypoxia with measured SpO_2 around 70% did not result in any significant deterioration in agreement between CO_{EPBF} and the reference methods. Furthermore, no systematic change in the bias was detected when moving to increasing degrees of hypoxemia.

The calculated mean percentage errors in study II were under all circumstances less than 25%. According to the a priori set 30% limit for maximum allowed mean percentage error, we therefore suggested that CO_{EPBF} can be considered to be equally effective as the reference methods CO_{TS} and CO_{Fick} when estimating CO during these conditions.

Trending ability during hypoxia

CO_{EPBF} showed good trending ability compared with CO_{TS} with a concordance rate of 86%. We decided to use a 10% exclusion zone for this comparison rather than the commonly recommended 15% zone.³⁷ The reason for this was the good calculated inherent precision of the reference technique. The choice of size of the exclusion zone is dependent on the inherent precision of the reference method. Simply put, the better the inherent precision, the smaller the exclusion zone. This is further discussed in the background section of this thesis. This principle was clearly illustrated when assessing the trending capacity of CO_{EPBF} against CO_{Fick} . We then found a comparatively poor concordance rate (81%), well below the generally accepted 92% limit.³⁷ The reason for this can most likely be attributed to the relatively poor inherent precision calculated for CO_{Fick} ($\pm 28\%$ for CO_{Fick} vs $\pm 6\%$ for CO_{EPBF}).

Impact of Pulmonary shunt in study I and II

It is important to take into consideration that the capnodynamic method estimates only the pulmonary blood flow that contributes to gas exchange, ie pulmonary shunt blood flow is not included. We did not determine the actual shunt in study II but in previous publications using an equivalent study setup, the shunt fractions have generally been in the region of 10 % ie relatively low.⁷²

In the experimental part of study I, pulmonary shunt fraction was calculated using Berggrens shunt formula⁸⁴ and was on average $12 \pm 2\%$ during baseline conditions PEEP 3 cmH₂O (data not published) ie corroborating previous results. In study II, where PEEP 5 cmH₂O was used throughout the protocol, it is therefore likely to assume that the shunt was of similar magnitude.

Laparoscopy- a “double hit” stress of the capnodynamic method

The main findings in study III was that PEEP needs to be set to a minimal level of 9 cmH₂O to preserve ventilation homogeneity, to ensure adequate VCO₂ and counteract increases in paCO₂ during CO₂ induced pneumoperitoneum in rabbits. Furthermore, PEEP 9 cmH₂O enabled continuous ELV based FRC monitoring with values and trending that matched well with the helium wash out reference method.

In previous studies ELV has been shown to closely reflect FRC under various circumstances. This has been validated against both tracer gas wash-out techniques^{11,71} and computed tomography¹⁰ as mentioned in the background section of this thesis.

Laparoscopic surgery is a clinically relevant scenario that from a theoretical point of view offers a double challenge for the capnodynamic estimation of FRC since it results both in a cranial shift of the diaphragm, ie reducing FRC, and simultaneously cause an increased CO₂ load, both due to the active insufflation of CO₂ in the abdominal cavity.^{85, 86} In clinical practice, these effects would likely be even more pronounced if laparoscopic surgery is performed in infants and small children, something that is becoming increasingly popular worldwide.⁸⁷

PEEP levels necessary to optimize ventilation during pneumoperitoneum

A variety of ventilation strategies have been suggested to optimize FRC and preventing hypoxia and hypercarbia during laparoscopy. In our study, we choose to investigate how various PEEP levels affect ventilatory conditions during this situation since we considered the use of PEEP the most theoretically appealing approach to counteract reduced FRC, atelectasis development and potential gas exchange disturbances. The effects of PEEP on the various ventilatory parameters in study III are discussed in detail below.

Effect on VCO₂ and LCI

At an IAP of 0 mmHg, paCO₂ was found to be relatively constant regardless of applied PEEP level. However, when exposed to an increased CO₂ load (and a cranial shift of the diaphragm) due to abdominal CO₂ insufflation, it is clear that PEEP 3 cm H₂O was insufficient to allow for adequate increase of VCO₂ in response to the increased CO₂ load. At IAP6 mmHg, both PEEP 6 cmH₂O and PEEP 9 cmH₂O were associated with increased VCO₂ whereas at IAP12 mmHg only PEEP 9 cmH₂O was capable of further increasing VCO₂, even if this increase was not statistically significant compared to PEEP 6 cmH₂O.

Furthermore, PEEP 9 cmH₂O was superior in maintaining LCI within normal values despite the increases of IAP, thus indicating better lung homogeneity.

The reason why PEEP 9 cmH₂O performed in a superior manner when compared to PEEP 3 cmH₂O and PEEP 6 cmH₂O regarding VCO₂ and LCI may be explained by at least two different but still interrelated factors. First, PEEP 9 cmH₂O is capable of keeping the lung constantly expanded, thereby avoiding both atelectasis formation and cyclic closing/reopening of the lung. This allowed for an adequate increase of VCO₂ in response to the increased CO₂ load when IAP was increased to 6 or 12 mmHg. Second, the increased abdominal pressure in itself (caused by CO₂ insufflation) will cause a cranial shift of the diaphragm, resulting in a decreased FRC if unopposed. By increasing PEEP the cranial shift of the diaphragm is counteracted, thereby better preserving FRC. Thus, both the more adequate expansion of the lung and the partial protection against too pronounced cranial shift of the diaphragm when using PEEP 9 cmH₂O help explain why this PEEP level appeared to be the best alternative to ensure satisfactory ventilation during pneumoperitoneum in this model of laparoscopic surgery in small infants.

Capnodynamic assessment of ELV during laparoscopy

ELV and EELV displayed an overall strong correlation with acceptable agreement for absolute values and good trending capacity when measured at PEEP 9 cmH₂O. No significant difference were seen between the absolute values of the methods for paired recordings done at 6 and 12 mmHg and the associated trending capacity was 100%.

Interestingly, PEEP 9 cmH₂O was also the PEEP level that appeared to offer best conditions for optimal lung homogeneity, as discussed above.

Since the capnodynamic technique is based on the measurement of exhaled CO₂, insufficient ventilation and excretion of CO₂ will negatively affect the performance of the method.^{10,11} It is therefore not surprising that the best correlation between ELV and EELV was found when the lungs were in a state of optimal homogeneity and CO₂ clearance.

It is important to emphasize that even if specific ELV values were found to numerically be very close to the corresponding EELV recordings (provided that the lung is adequately expanded), ELV should not be viewed as synonyms to EELV.⁷¹ This is because the two parameters strictly speaking refer to two different volumes, even though these volumes clearly are closely related. EELV represents an anatomical volume whereas ELV quantifies the lung

volume participating in gas exchange including the airway volume up to the site of the CO₂ sensor and the carbon dioxide dissolved in lung tissue and lung capillary blood.¹¹ It can therefore be challenging to strive for numerically perfect coherence between the methods, even when recordings are done under optimal conditions with regards to lung homogeneity. However, it must be considered a minimal requirement that ELV and EELV should display values in the same order of magnitude and offer excellent trending capacity, in order for ELV to be considered reliable in the clinical context. In study III, these requirements were met only with PEEP 9 cmH₂O and it is therefore this level, that we suggested as most favorable for both optimal ventilation and for the performance of the capnodynamic method in estimating FRC during CO₂ pneumoperitoneum.

Hemodynamic effects of high PEEP

One common concern is that application of high PEEP would result in reduced venous return, potentially affecting CO and MAP negatively. The fact that increased PEEP has circulatory effects on children is well established.^{88,89,90,91} but the extent of this impact on haemodynamics is difficult to predict and is dependent on the underlying circulatory status of the patient. In study I, we showed that increasing PEEP from 3 to 10 cmH₂O in anaesthetized mechanically ventilated infants, will only affect CO minimally (in the region of 10%, data not published).⁷⁰ This indicates that PEEP levels around 10 cmH₂O has minimal effects on CO and MAP, at least in healthy infants.

In support of this, in study III the application of PEEP to 9 cmH₂O during pneumoperitoneum and elevated IAP, was not associated with any hemodynamic alterations, as demonstrated by a maintained mean arterial pressure during the increase of PEEP from 3 via 6 to 9 cmH₂O. Thus, the use of a comparatively high PEEP level in this context, did not result in any negative circulatory effects.

Pulmonary shunts during laparoscopy

Pulmonary shunt fraction was not measured directly in study III, which is a limitation of the protocol. In this study however, FRC as measured with the gold standard EELV at PEEP 9 cmH₂O, was between 15 and 34 ml kg⁻¹ which is close to the normal range of EELV for most mammals (ie approximately 25 ml kg⁻¹), thus at least theoretically ensuring an adequately open lung with minimal atelectasis and shunting.⁹² Furthermore, lungomogeneity, as measured by LCI, was superior at PEEP 9 cmH₂O which indicates an evenly ventilated and open lung, with a high probability of minimal shunting.

LIMITATIONS

The various limitations associated with the thesis studies have been addressed in the Background and Discussion sections of this thesis as well as in the separate manuscripts. However, it is of special importance to highlight the following more fundamental limitations of the dynamic capnography method:

1. Even if dynamic capnography now has been applied and validated in humans for the first time, further studies are needed for confirmation of accuracy and to prove its usefulness in the clinical setting.
2. The lack of a true gold standard for CO measurements remains a restriction for the validation of any new CO method, included CO_{EPPBF} .
3. The definition of an acceptable agreement between a tested method and the reference method is currently set to a mean percentage error of less than 30%. This is, however, debatable and the importance of inherent precision for methods should be considered when judging the agreement.
4. The studies are performed on three different species, humans, piglets and rabbits, making it difficult to compare results between the studies and challenging to draw overall conclusions.
5. Dynamic capnography does not account for pulmonary shunted blood flow. Despite the use of established recruitment methods, we have no radiographic verification of the level of lung expansion, which must be taken into account when interpreting the study results.
6. Under most circumstances ELV and FRC/EELV are closely related. However, they do represent two different entities, one anatomical and one functional, making the interpretation of their numerical conformity challenging.

FUTURE PERSPECTIVES

This thesis has evaluated dynamic capnography in a structured manner in accordance with the current consensus for CO and FRC validation studies, with encouraging results. The next natural step in the development is to further explore the method's limitations and usefulness in clinical contexts. Clinical observational studies and multicenter randomized controlled outcome studies are appropriate strategies for assessing the clinical benefit of the method. Introduction of the method in the intensive care environment would provide an interesting addition to this.

ELV has been shown to adequately reflect FRC in most situations. Both in the anaesthetic and intensive care environment, this can be used for studies on individually adjusted ventilation maneuvers with ELV as an endpoint of lung expansion. Again, clinical observational and outcome studies have to be undertaken to evaluate this.

In the laboratory setting, DC needs to be further investigated for its limitations during situations of abnormal physiology. From a paediatric perspective, an important and relatively common scenario is intra and extra cardiac shunting. In addition, single ventricle physiology and estimation of pulmonary blood flow during persistent pulmonary hypertension of the newborn would potentially be of value in the care of children with congenital heart disease and compromised lung circulation.

Dynamic capnography allows for reliable estimation of some the parameters in Fick's classic CO formula, thus, opening up mathematical derivation of the other parameters. Examples of this are mixed venous oxygen saturation (SvO_2) and shunt fraction estimations (both pulmonary and circulatory shunts). Preliminary animal studies using invasive mixed venous blood gas sampling as reference method has already shown promising features.

7 CONCLUSIONS

During the various challenges performed in the studies, capnodynamic estimation of CO and EELV were found to compare well with established comparative techniques.

Specific conclusions

Study I

In the first human application of capnodynamic CO_{EPBF} we conclude that the method is clinically feasible.

In a porcine model resulting in moderate CO changes, CO_{EPBF} showed excellent agreement for absolute values and trending ability compared with the gold standard CO_{TS} .

Against the findings of the porcine study, CO_{EPBF} appears more reliable compared to the current clinical standard, CO_{SSD} with regards to the ability to detect moderate changes in CO.

Study II

CO_{EPBF} show good agreement for absolute values and trending ability when compared to gold standard CO_{TS} in a setting of selective hypoxic pulmonary vasoconstriction.

Study III

In a model of paediatric laparoscopic surgery, a PEEP level of 9 cmH₂O is required to preserve FRC, ventilation homogeneity and allow for adequate CO₂ clearance.

ELV was found to adequately reflect FRC with good agreement of absolute values as well as good trending ability when compared against helium wash-in/wash-out provided sufficient lung homogeneity.

8

ACKNOWLEDGMENTS

The completion of this thesis would not have been possible without the contribution from all the amazing people that have helped me on the way. Here they are,

Per-Arne Lönnqvist, my main supervisor. Thank you for taking me on board this exciting endeavor! You possess the enviable and impressive feature of being a Professor, while at the same time enjoy chatting about football over a pint. You have always struck me as a man who puts the bible on the floor!

Per Winberg, co-supervisor, for your outstanding experience and knowledge of paediatric cardiology in general and cardiac ultrasound in particular! You have provided excellent help many times, both in research and in real situations with patients. Your cardiac echo knowledge is impressive! Thank you for all your help with Doppler measurements! Your attention to details has been especially useful!

Walid Habre, co-supervisor, for your help with so many things! Not only are you an exceptional researcher and clinician, you are also a down to earth person with a very warm character! Thank you for being such an awesome host when we did our experiments in the lab in Geneva! I really hope I will be back in your lab some day.

Jonas Berner, head of the Department, for always supporting my research and for allowing my sabbatical leave so I could complete a clinical fellowship in the UK.

Peter Larsson and ***Katarina von Schewelov***, my clinical directors for supporting my projects and giving me time off for research.

Mats Wallin, you are an outstanding scientist full of excellent ideas! You have also the rare capability of transforming them into actual research and publications! Also very impressed of how creative and hands on you are in the lab!

Magnus Hallbäck, for so many things! I really owe a lot to you for this thesis. Thank you for always taking a step back and double check the maths and statistics to see if my enthusiasm over results was justified....

Bronwyn Scarr for excellent help in study I and for proof reading.

Gergerly Fodor, for help with designing and conducting the experiments in Geneva. Awesome job! Also very good critical proof reading of my manuscript for that study!

Andre dS Rocha and *Na Lin* for excellent proof reading and ideas for study III.

Gergerly Albu and *Sam Bayat* for letting me see your fascinating research at the particle accelerator, European Synchrotron Radiation Facility ESRF, in Grenoble and for teaching me the helium wash in/wash-out technique there.

Anders Larsson and *Miklós Lipcsey* at Hedenstierna lab for excellent help and expertise in animal research.

All technicians at The Hedenstierna lab for help with the animals and experience in conducting animal research.

Staff and directors at the lab in Geneva for help with animal handling of the rabbits used in the studies there. Special thank to lab technician Xavier Belin for outstandingly skilled help with the animal preparation!

Plastic surgeons *Erik Neovius* and *Petra Petersson* for help with patient recruitment.

Anders Modin, my external mentor, for excellent support and discussions!

Lennart Christiansson, clinical mentor and Director of studies, when I was a registrar in Uppsala. You encouraged me to think in a critical and structured way. You once said that “just because someone hasn’t got a PhD, it does not mean that they are free from responsibility to think scientifically”. A philosophy that has inspired me a lot!

All *anaesthetists and intensivists at BANE/BIVA!*

Martin Räf, my clinical scheduler for the last 8 years. I honestly can’t remember one single occasion when you haven’t done all in your power to meet my requests for research time (or skiing).

Urban Fläring for hiring me back in 2011. Also Urban, thank you for showing me the statistical software that I’ve used. It was an absolute game changer! I am absolutely certain that it saved me many months!

All *anaesthetic nurses at BANE* for helping me with the clinical research!

All *theatre staff at BANE* for letting me sit with my laptop in theatre and write a large part of this thesis there!

Secretaries *Lena Centervall* and *Lena Pålsson* for helping me with most of my administration. I promise I will try to get better in that area!

Ulf Johansson for layout and for making this collection of words actually look like a book.

Colleagues at the Anaesthetic Departments at Gävle District General Hospital and Uppsala University Hospital, for many great years when I was a registrar in anaesthesia and intensive care.

Colleagues at Birmingham Children's Hospital for introducing me to the world of paediatric anaesthesia

Former *fellow colleagues and consultants at The Freeman Hospital Children's Heart Unit* in Newcastle for teaching me paediatric cardiac anaesthesia! I can't see any better way to prepare for this thesis than going through the outstanding training I got there!

My *parents Lena and Göran* for all your support and love throughout the years and for making this possible!

My *siblings Ella, Axel, Henrik and Love*. You have always been a huge inspiration and motivation!

My wonderful kids *Åke, Linus and Emma*. I love you so much!

My dear *Malin*, you are an absolutely incredible partner and the love of my life. I love you!

This work was supported by the Karolinska Institute and partially by unrestricted grants from Maquet Critical Care AB, Solna Sweden, and the regional agreement on medical training and research (ALF) between Stockholm County Council and the Karolinska Institute.

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