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Measurement of carbon monoxide transfer and lung volume in ventilated subjects

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ABSTRACT: A simple method for measuring lung volume and carbon monoxide transfer factor (TLCO) by a rebreathing technique was assessed in nine healthy volunteers undergoing intermittent positive pressure ventilation (IPPV). Measurements of TLCO, alveolar volume (V_A) and carbon monoxide transfer coefficient (KCO) made at three inspired oxygen concentrations (21, 35 and 70%) during IPPV were compared to those obtained during spontaneous breathing. The effects of 10 cmH_2O positive end expiratory pressure (PEEP) were also studied. Pulmonary capillary blood volume (Vc) and the diffusing capacity of the alveolar capillary membrane (Dm) were derived.

There was a close correlation between measurements of TLCO during IPPV ($TLCO_{IPPV}$) and spontaneous breathing ($TLCO_{SV}$) (r=0.92). Ventilated TLCO was 64±8% of spontaneously breathing TLCO. There was a close agreement between ventilated and spontaneously breathing measurements of KCO (r=0.95; mean difference 0.14, 95% limits of agreement +0.37 to -0.09 $mmol \cdot min^{-1} \cdot kPa^{-1} \cdot l^{-1}$). Vc was 92±23 ml during spontaneous breathing and 72±21 ml during IPPV ($p<0.05$). PEEP of 10 cmH_2O significantly increased functional residual capacity (2.3±0.5 to 3.5±0.6 *l*) and decreased TLCO (5.9±1.0 to 5.3±1.2 $mmol \cdot min^{-1} \cdot kPa^{-1}$), KCO (1.7±0.2 to 1.1±0.3 $mmol \cdot min^{-1} \cdot kPa^{-1} \cdot l^{-1}$) and Vc (82±22 to 56±20 ml). Dm did not change with PEEP.

This simple method may be a useful means of assessing gas exchange and lung volume in ventilated subjects. It showed that PEEP increased lung volume but reduced TLCO and that this reduction appeared to be due to a reduction in capillary blood volume.

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The assessment of respiratory function in patients ventilated for respiratory failure is frequently rudimentary and often limited to arterial blood gas analysis [1]. This deficiency has resulted in little being known of the natural history of the disordered physiology of the adult respiratory distress syndrome and other serious pulmonary disorders. Furthermore, assessing the effects of existing and new therapeutic interventions in such cases has proved difficult. This is in marked contrast to the thorough evaluation of pulmonary function possible in ambulant patients with less severe respiratory impairment. Thus, the measurement of pulmonary carbon monoxide transfer capacity (TLCO); or carbon monoxide diffusing capacity, (DLCO) is commonly used as a simple method of assessing efficiency of pulmonary gas exchange. TLCO could be a useful method of assessing patients with acute respiratory failure requiring positive pressure ventilation, but few descriptions of methods for measuring TLCO in ventilated subjects have been published. Those techniques that have been described have been complicated and difficult to use at the bedside. We therefore adapted a standard laboratory rebreathing method for measuring TLCO and accessible lung volume, such that it could be undertaken on ventilated subjects at the bedside using equipment readily available in any respiratory function laboratory. This paper describes the technique and the comparison of measurements made in normal volunteers whilst spontaneously breathing and during positive pressure ventilation. We also used the method to assess the effects of positive pressure ventilation, with and without 10 cmH_2O positive end expiratory pressure (PEEP), on lung volume and gas exchange in normal subjects.

Methods

Nine healthy male volunteers (age range 25–40 yrs, 1 smoker) who gave their informed consent were recruited. The study was approved by the Ethics

Committee of the Royal Brompton National Heart and Lung Hospital.

Rebreathing measurement

A laboratory method for the measurement of TLCO by rebreathing was adapted for use in ventilated patients [2]. In brief, a rebreathing bag with an attached two-way valve was filled from a calibrated syringe with a measured volume of gas comprising helium (He) 14%, carbon monoxide (CO) 0.3%, oxygen (O_2) either 21%, 35% or 70%, and balance nitrogen (N_2). The initial concentrations of these gases were measured with a combined katharometer and infra-red analyser (PK Morgan, Kingston, Surrey, UK) and an anaesthetic respiratory gas monitor (5250 RGM, Ohmeda, Hatfield, Herts, UK). At the end of a full expiration to residual volume (RV) when spontaneously breathing, or at the end of a normal expiration (functional residual capacity (FRC)) when ventilated, the valve was switched to the rebreathing position. Rebreathing continued for a timed period of six breaths (approximately 15 s during spontaneous breathing, 25 s if ventilated), ensuring that the bag emptied completely with each inspiration. The duration of rebreathing was taken from the moment of valve opening until the valve was closed at the end of the sixth expiration. The concentrations of He, CO, O_2 and CO_2 in the breathing bag were then measured. The end expiratory He and CO concentrations were corrected for changes in O_2 and CO_2 concentrations which occurred during the rebreathing test. To estimate "CO back pressure" (CO_{BP}) a 2 ml venous blood sample was obtained immediately prior to each series of measurements and 10 min after the completion of the last test. Haemoglobin (Hb) and carboxyhaemoglobin (COHb) levels were measured simultaneously by co-oximetry (Corning 2500, Braintree, Essex, UK).

All measurements were undertaken with the subjects in the supine posture. Two different bag volumes were used; 80% of supine vital capacity for spontaneously breathing measurements (*i.e.* about 3,500 ml) and 1,000 ml for ventilated measurements. This rather small volume was chosen for measurements in ventilated subjects as it was predicted that this would be the largest volume that would be tolerated by most patients with lung disease undergoing mechanical ventilation.

Bag-in-box system

A bag-in-box system was designed and built, to permit the application of these rebreathing manoeuvres to ventilated subjects without influencing set ventilatory pressures, such as the level of PEEP. The system was placed in the breathing circuit between patient and ventilator (fig. 1). A single manually-operated valve switched between conventional ventilation and rebreathing mode. The box contained a 1 *l* rubber anaesthetic bag with a filling port on the valve block. Each subject was trained to tolerate positive pressure ventilation (Dräger Evita, Dräger, Hemel Hemstead, Herts, UK) *via* a mouthpiece, whilst wearing a noseclip. The ventilator was set to deliver a tidal volume of 1,000 ml, at a rate of 15 breaths·min^{-1}, and with an inspiratory to expiratory time ratio of 1:1.5. The rebreathing bag was filled and placed in the respiratory circuit as described above. After a period for stabilization, the valve was switched at the end of a tidal expiration (FRC) and rebreathing continued for six breaths as described above.

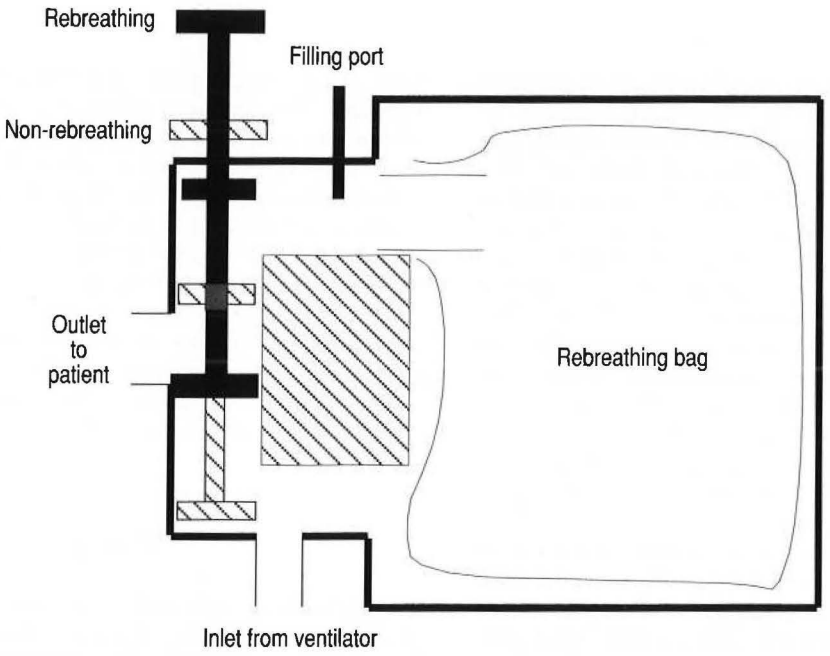

Fig. 1. – Bag-in-box system.

Experimental protocols

After an initial rest period of 10 min, measurements of T_{LCO} were undertaken at intervals of 7.5–10 min, in order to facilitate adequate wash-out of alveolar CO and He between tests. Each result represented the mean of two measurements, which were repeated if there was a greater than 10% difference between them. Two series of experiments were undertaken.

Experiment 1

A series of paired measurements was made to compare results obtained from the standard spontaneously-breathing laboratory rebreathing technique with those from the bag-in-box system in ventilated subjects. In order to study a range of gas transfer values in normal volunteers, measurements of accessible lung volume (V_A) and T_{LCO} were undertaken by seven of the subjects, spontaneously breathing and ventilated using gas mixtures containing O_2 concentrations of 21, 35 and 70%. In the other two subjects, measurements were undertaken with 21% O_2 only. Subjects breathed the equivalent fractional inspired oxygen concentration (F_{IO_2}) *via* a non-rebreathing circuit for 3 min prior to each measurement. Each pair of measurements was made on separate days and in identical conditions.

By measuring T_{LCO} at three different oxygen concentrations, values could be derived for the two components which contribute to the transfer of carbon monoxide, diffusion across the alveolar capillary membrane (Dm), and the chemical reaction with the blood (θVc, where θ=reaction constant for combination of carbon monoxide with haemoglobin; and Vc=volume of pulmonary capillary blood) [3]. Values for Dm and Vc were obtained for seven subjects while breathing spontaneously, and while undergoing mechanical ventilation.

Experiment 2

The effect of PEEP on lung volume and T_{LCO} was assessed in eight ventilated normal subjects. Measurements were made at 0 and 10 cmH_2O of PEEP, the level of which was confirmed from the displayed airway pressure. For each level of PEEP, measurements were undertaken at three different levels of F_{IO_2} (21, 35 and 70%). The order of measurements was randomized. Lung volume, T_{LCO}, Vc and Dm were derived for each level of PEEP.

Calculations

The alveolar volume (V_A), end expiratory volume (either FRC or RV depending on the rebreathing manoeuvre undertaken), T_{LCO} and carbon monoxide transfer coefficient (K_{CO}) were calculated using standard equations [4] corrected for CO_{BP}. The COHb concentration during each test was calculated using the following formulae:

$$COHb_x = COHb_{initial} + x\,\frac{(COHb_{final}-COHb_{initial})}{n}$$

Where x = test number and n = total number of tests undertaken between measurements of COHb. CO_{BP} was then derived as predicted by the Haldane relation [5]:

$$CO_{BP} = \frac{P_cO_2 \times COHb}{225 \times HbO_2}$$

Where P_cO_2 (mean capillary oxygen tension) was estimated to be equal to the end expiratory value - 1.5% and HbO_2=1-COHb. T_{LCO} was corrected to a haemoglobin of 14.6 $g \cdot dl^{-1}$ according to the method of COTES *et al.* [6].

From ROUGHTON and FORSTER [3], T_{LCO} can be expressed as the sum of two conductances:

$$\frac{1}{T_{LCO}} = \frac{1}{Dm} + \frac{1}{\theta Vc}$$

θ is derived from the Hb and the alveolar oxygen partial pressure, according to the following formulae [3]:

$$\frac{1}{\theta} = \frac{0.33+0.0057 \times P_cO_2}{(Hb/14.6) \times (1-COHb)}$$

Dm and Vc were then obtained from the y axis intercept and the slope, respectively, when $1/T_{LCO}$ was plotted against $1/\theta$.

Statistical methods

Results are shown in the text and figures as mean ±standard deviation. Correlation between measurements was assessed using linear regression analysis. The mean difference between ventilated and non-ventilated measurements and the 95% limits of agreement were calculated for K_{CO} and T_{LCO} [7]. Student's paired t-test was used to analyse changes in the measured parameters following the application of PEEP. A value of $p<0.05$ was considered to be significant.

Results

A total of 23 paired measurements of T_{LCO} and K_{CO} in nine subjects was made to compare the spontaneously breathing and ventilated methods. Ventilated T_{LCO} ($T_{LCO_{IPPV}}$) was 64±8% of spontaneously breathing

T_{LCO} ($T_{LCO_{SV}}$), but there was a significant correlation between the two (r=0.92, p<0.001) (fig. 2). There was also a significant correlation between ventilated Kco ($K_{CO_{IPPV}}$) and spontaneously breathing Kco ($K_{CO_{SV}}$) (r=0.95, p<0.001) (fig. 3). $K_{CO_{IPPV}}$ tended to be higher than $K_{CO_{SV}}$; the mean difference between the two being 0.14 (95% limits of agreement +0.37 to -0.09) mmol·kPa^{-1}·min^{-1}·l^{-1}.

In seven subjects, Dm and Vc were derived from measurements of $T_{LCO_{SV}}$ and $T_{LCO_{IPPV}}$ made at three different values of F_{IO_2}. Mean Vc was 92±23 ml during spontaneous breathing and 72±21 ml (p<0.05) during mechanical ventilation. Dm measured during spontaneous breathing and during mechanical ventilation was 14.3±1.7 and 8.1±1.1 mmol·min^{-1}·kPa^{-1}, respectively (p<0.001).

The application of 10 cmH_2O of PEEP caused a rise in mean functional residual capacity (FRC) (2.3±0.5 to 3.5±0.6 *l*, p<0.001). T_{LCO} fell from 5.9±1.0 to 5.3±1.2 mmol·kPa^{-1}·min^{-1} (p=0.01) and Kco fell from 1.7±0.2 to 1.1±0.3 mmol·min^{-1}·kPa^{-1}·l^{-1} (p<0.001).

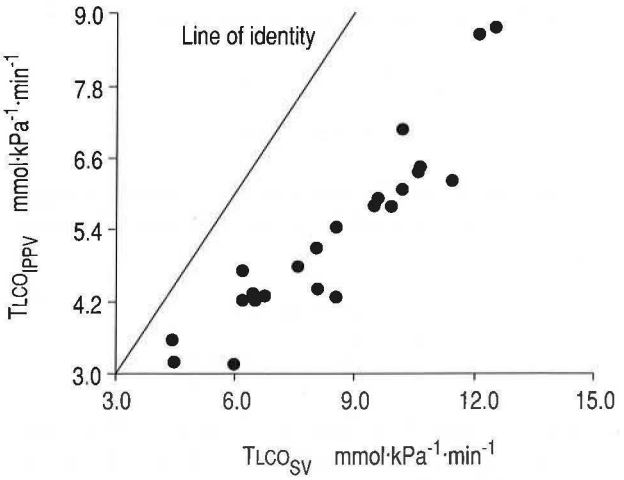

Fig. 2. – T_{LCO} spontaneously breathing ($T_{LCO_{SV}}$) plotted against T_{LCO} ventilated ($T_{LCO_{IPPV}}$); $T_{LCO_{IPPV}}$=0.598·$T_{LCO_{SV}}$+0.28; r=0.92; p<0.001. T_{LCO}: transfer factor of the lungs for carbon monoxide.

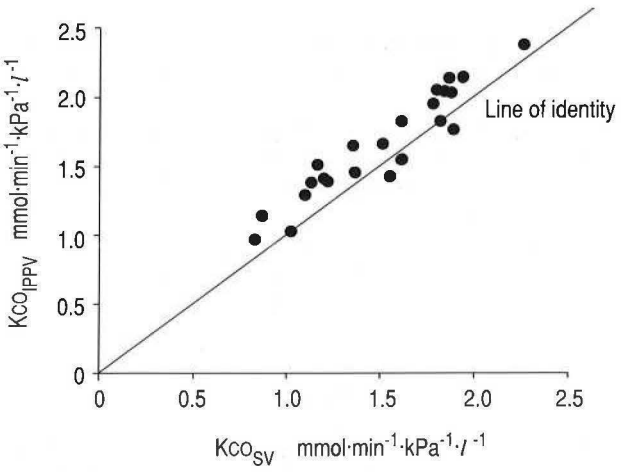

Fig. 3. – Kco ventilated ($K_{CO_{IPPV}}$) plotted against Kco spontaneously breathing ($K_{CO_{SV}}$); $K_{CO_{IPPV}}$=0.919·$K_{CO_{SV}}$+0.26; r=0.95; p<0.01. Kco: transfer coefficient for carbon monoxide.

The mean Vc fell from a baseline value of 82±22 to 56±20 ml following the application of PEEP (p<0.05). There was a significant correlation between the change in T_{LCO} and the change in Vc for individuals (r=0.71, p<0.05). PEEP had no effect on Dm; 7.5±1.0 mmol·min^{-1}·kPa^{-1} without PEEP and 7.2±1.5 mmol·min^{-1}·kPa^{-1} with 10 cmH_2O PEEP (p=0.27).

Discussion

We have shown that T_{LCO} and lung volume can be measured in ventilated subjects using a simple rebreathing technique, and that PEEP results in an increase in V_A associated with a reduction in T_{LCO}.

Although T_{LCO} can be measured using a number of methods, the single breath technique is the commonest in routine clinical use. However, the rebreathing method would appear to be the most appropriate for undertaking measurements in ventilated subjects for a number of reasons. Firstly, the single breath technique is likely to be influenced to a greater extent by the marked inhomogeneity of gas distribution frequently encountered in the critically ill, when it would underestimate the true value of T_{LCO}. Secondly, it is much easier to develop a rebreathing method which is sterile and reduces the risks of cross infection [2]. Thirdly, a close relationship between rebreathing and single breath measurements has been demonstrated [2]. Finally, the single breath method would probably be poorly tolerated by critically ill subjects, who may be unwilling or unable to breathhold for 10 s.

In calculating T_{LCO} from only two measurements of CO concentration, we assumed that the lung is a single compartment and that instant gas mixing occurs throughout the compartment, such that the elimination of CO follows a single exponential pattern. These assumptions result in the measured value of T_{LCO} underestimating the true value. It has been proposed that CO uptake during rebreathing should be considered as a two-compartment model [8], described mathematically as a biexponential process; a fast component representing distribution between the rebreathing reservoir and alveolar compartment, and a slow component representing uptake into the pulmonary capillary blood. However, in order to measure T_{LCO} using this analysis, continuous measurements of CO concentrations are necessary. Mass spectrometry and the stable isotope of CO, $^{12}C^{18}O$, are needed as the abundant species of CO ($^{12}C^{16}O$) and atmospheric nitrogen have the same mass:charge ratio and are, therefore, indistinguishable [9]. As making these measurements is not feasible in the majority of intensive care units, we assessed this simpler method of measuring T_{LCO} using equipment readily available in most centres, whilst recognizing the limitations and potential errors of the technique.

A second assumption made, is that the system volume (bag+lung) remains constant during the period of rebreathing. The uptake of CO and the highly insoluble He can be ignored due to the very small

volumes involved. The increase in CO_2 concentration of about 4% during the period of rebreathing is matched by a similar fall in O_2, reflecting a respiratory quotient of approximately 1. The system volume, therefore, remains essentially constant using the gas mixture employed in this study. However, as the analysers used to measure CO and He have a soda lime filter to remove any CO_2, the post-rebreathing measurements must be corrected for this loss. Finally, in order to simplify the calculation of TLCO the effective rebreathing rate is assumed to be infinity. In reality, by using a rate of 15 breaths·min^{-1} the true value of TLCO tends to be underestimated. However, the use of higher respiratory rates in ventilated subjects generated unacceptably high airway pressures and may also have adverse effects on cardiac output.

$TLCO_{IPPV}$ correlated closely with $TLCO_{SV}$, although the former tended to be approximately 60% of the self-ventilating value. This reflects the fact that ventilated measurements were made at a mean alveolar volume of 3.22 *l* compared with 5.69 *l* for $TLCO_{SV}$. It is well-known that TLCO tends to fall as VA drops [10, 11]. This may reflect a true fall in the efficiency of gas exchange, due to a reduction in Vc and alveolar surface area, combined with impaired matching of ventilated to perfused alveoli at low lung volumes. In addition, it may be an artifact due to an increased dead space:tidal volume ratio, such that when a small volume is inhaled a larger proportion of the gas mixture is in contact with a non-gas exchanging surface.

There was a close agreement between the ventilated and spontaneously breathing values for KCO, the former being only 0.14 mmol·min^{-1}·kPa^{-1}·*l*$^{-1}$ greater than the latter. Measured KCO increases if estimations are made at a small alveolar volume [10, 11] as Vc decreases less than the fall in lung volume. It might have been predicted that KCO_{IPPV} would be considerably larger than KCO_{SV}, as the ventilated measurements were undertaken at a smaller VA. However, raised intrathoracic pressure during positive pressure ventilation reduces KCO by means of a fall in Vc [12]. These effects would appear to cancel each other out, such that KCO_{IPPV} and KCO_{SV} agree closely with each other. The close correlation between the ventilated and spontaneously breathing measurements implies that when TLCO and KCO are measured by this technique, they reflect the same changes in lung function and respond to the same influences as the more conventional measurements.

The technique of assessing Dm and Vc by measuring TLCO at different values of FIO_2 was first described by ROUGHTON and FORSTER [3]. It has been shown to be both accurate and reproducible, and has been used to assess change in Vc in a variety of pathological states including emphysema [13], rheumatoid arthritis [14], and pulmonary embolism [15]. The mean value of 92 ml obtained for Vc measured during spontaneous respiration is similar to values reported in other studies of normal subjects. The mean value of 14.3 mmol·min^{-1}·kPa^{-1} for Dm in this study is at the lower end of the range reported by others using the same technique, and is lower than the result obtained using a recently described technique for the simultaneous measurement of CO and nitric oxide (NO) transfer factors [16]. The exact value of Dm obtained by the technique used in the current study is very dependent on the value of θ (a composite rate constant for the combination of carbon monoxide with haemoglobin within the erythrocyte). A number of different values have been described which may explain some of the differences seen in estimates of Dm between studies. Furthermore, Dm is calculated from the y intercept of a regression line and is likely to have a greater error than the measurement of Vc represented by the slope of the same line.

The theory behind measuring Vc and Dm using different values of FIO_2 assumes that the haemoglobin is almost fully saturated along the length of the pulmonary capillary, such that each molecule has only one potential binding site for CO (*i.e.* the haemoglobin is either HbO_3 or HbO_4). As the value of θ depends upon the number of oxygen molecules bound to haemoglobin [4], under these conditions it can be assumed to be constant. In theory, measurements using this technique should only be undertaken using gas mixtures containing more than 28% oxygen [5], when it can reasonably be assumed that only HbO_3 or HbO_4 are present. However, a number of previous studies have utilized measurements with a FIO_2 of 21% and have still obtained reasonable values for Vc and Dm [13–15]. Moreover, we found no significant difference between Vc and Dm calculated from measurements made in this study using 35 and 70% oxygen only, or from those values derived when all three oxygen levels were used.

Mean Vc during IPPV was significantly lower than during spontaneous ventilation. This may reflect the increase in intrathoracic pressure during IPPV, resulting in a reduced capillary volume, or may reflect the lower VA at which the ventilated measurements were made. The large reduction in Dm measured during IPPV reflects the large differences in VA between ventilated and spontaneously breathing measurements.

By employing a bag-in-box system, we were able to undertake measurements without interfering with the set ventilator pressures and were, therefore, able to assess the effects of PEEP. The application of 10 cmH_2O of PEEP caused a highly significant increase in FRC in all subjects. Assuming that the subjects had a normal total thoracic compliance of around 100 ml·cmH_2O^{-1}, the observed mean increase in FRC of 1.1 *l* was as predicted. PEEP also caused a significant fall in $TLCO_{IPPV}$ in all but one subject studied. When combined with the increase in lung volume, this caused KCO to fall in all subjects. The subject who failed to show a fall in TLCO with PEEP was the only smoker studied, and may have had ventilation: perfusion mismatch which was reversed with PEEP. PEEP caused a significant fall in Vc but had no effect on Dm. As there was a positive correlation between the change in TLCO and change in Vc, the effect of PEEP on TLCO was probably due to a fall in

Vc. Dm results were much more variable (see above) and no clear trend emerged following the application of PEEP.

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