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Measurement of lung volume in mechanically ventilated monkeys with an ultrasonic flow meter and the nitrogen washout method

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Abstract *Objective:* Measurement of functional residual capacity (FRC) during mechanical ventilation is important to standardise respiratory system compliance and adjust the ventilator settings to optimise lung recruitment. In the present study we compared three methods to measure FRC. *Design:* The bias flow nitrogen washout technique (FRC_{N₂MC}), the multiple breath nitrogen washout (FRC_{MBNW}) and the multiple breath sulphur-hexafluoride washout using the molar mass signal of an ultra-

sonic flow meter (FRC_{MBSF₆}) were compared in six adult monkeys after endotracheal intubation and during spontaneous breathing and mechanical ventilation at three different positive end-expiratory pressure (PEEP) levels of 0, 5 and 10 cmH₂O.

Setting: Animal research laboratory.
Results: We found good agreement between all three methods and they all accurately measured changes in FRC when PEEP was increased.

The coefficients of variance of the three measurement techniques were in the same range (1.3–9.2%).

Conclusion: The measurement of the tracer gas concentration with the molar mass signal of the ultrasonic flow meter provides a good and simple alternative to respiratory mass spectrometer for FRC measurements in ventilated subjects.

Keywords Functional residual capacity · Mechanical ventilation · Positive end-expiratory pressure

Introduction

Functional residual capacity (FRC) is an important lung volume without which lung mechanics such as dynamic lung compliance and resistance are difficult to interpret during mechanical ventilation [1]. The empirical titration of positive end-expiratory pressure (PEEP) to optimise lung volume and, hence, lung recruitment in diseased lungs is usually based only on clinical judgement such as blood gas analysis, chest expansion on X-ray and the interpretation of pressure-volume loops [2].

There are several techniques available to measure FRC in mechanically ventilated patients: the closed-circuit helium dilution technique, the open-circuit nitrogen washout or the open-circuit sulphur-hexafluoride (SF₆) wash-in/washout. The helium dilution technique is very cumbersome and sensitive to leaks in the ventilatory system [3]. Nitrogen washout techniques can be divided into bias flow and breath-by-breath washout systems. The bias flow nitrogen washout method uses a mixing chamber (MCN2), whereas the multiple breath nitrogen washout (MBNW) technique measures the instantaneous

nitrogen concentration and air flow during each breath at the airway opening. MCN2 allows very accurate measurement of FRC [4] but is limited to patients at FiO_2 less than 0.7 since, for accurate volume measurement, the technique requires a change in the tracer gas (N_2) of more than 0.3 [5]. Recently, newer techniques using sulphur hexafluoride (SF_6) as a tracer gas have been developed [6, 7, 8, 9]. SF_6 is an inert gas which is fed into the inspiratory limb of the ventilator circuit. The concentration of SF_6 is measured breath-by-breath using a respiratory mass spectrometer [10] or an infrared analyser [8], the former having a high, and the latter having a low, signal-to-noise ratio. Alternatively, the SF_6 concentration can be measured by the molar mass signal (MM) of an ultrasonic flowmeter at the same time as flow (and hence volume) is recorded. The molar mass method has been shown to be highly accurate and reproducible in a mechanical lung model [11] and sensitive to changes in FRC secondary to alterations of positive end-expiratory pressure (PEEP) in ventilated rabbits [6] and in spontaneously breathing healthy infants [7].

The aim of the study was to compare the molar mass method (FRC_{MM}) with the bias flow nitrogen washout technique using a mixing chamber (FRC_{MCN2}) and also with the multiple breath nitrogen washout (FRC_{MBNW}) in mechanically ventilated adult rhesus monkeys.

Methods

Subjects

Six adult rhesus monkey with a mean body weight of 10.9 ± 2.7 kg were studied. Analgesia for intubation was achieved intramuscularly with ketamine (40 mg/kg) and for the duration of the experiment anaesthesia was maintained with intravenous infusion of thiopentone sodium (8.0 mg/kg per h). After oral intubation of the trachea, the cuff was inflated sufficiently to prevent any air leak detectable by auscultation when the lungs were inflated to +40 cmH₂O pressure. The animals breathed spontaneously for the first set of experiments and were mechanically ventilated at modest settings to keep the $PETCO_2$ in the 35–40 mmHg range in the second set. The animals were placed supine. Heart rate was monitored with a three-lead continual electrocardiogram (Sirecust Model 1281, Siemens, Erlangen, Germany) and transcutaneous haemoglobin saturation (SpO_2) was obtained with pulse oximetry (Nellcor Type B, Hayward, CA). Animals were maintained in an in-house colony at Novartis, Basel, Switzerland, under the full-time care of an experienced primate veterinarian and his staff. All experimental protocols conformed to international standards of animal welfare and were approved and periodically reviewed by the Kantonale Tierversuchs-Kommission von Basel-Stadt und Basel-land, Switzerland.

Equipment

The experimental set-up is shown in Fig. 1. Two ventilators of the same manufacturer with the same settings were used during the measurements (Hamilton Medical, Rhäzuns, CH). In order to perform the nitrogen washout, a sliding valve triggered by a computer was used to switch the monkeys from the first ventilator ($FiO_2 =$

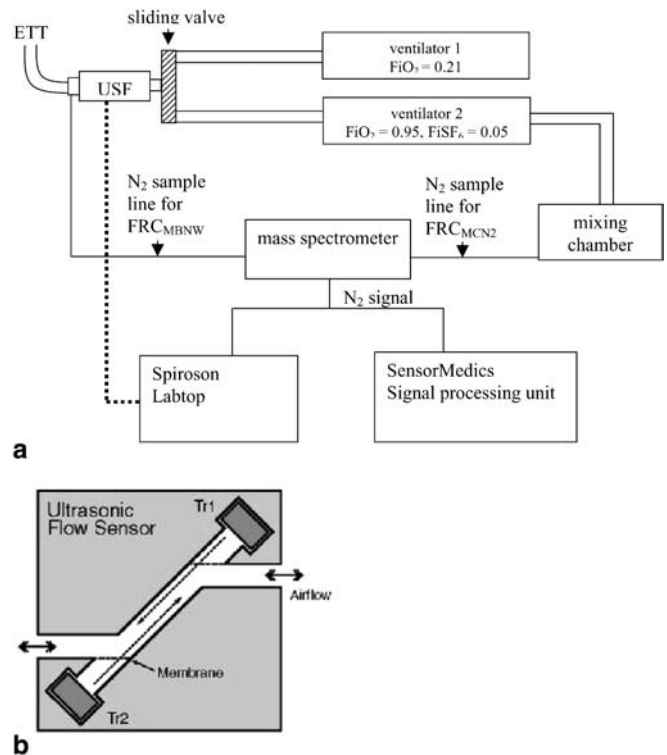


Fig. 1 a Experimental set-up of functional residual capacity (FRC) measurements b Design of the ultrasonic flowmeter $Tr1 =$ transducer one, $Tr2 =$ transducer two, of the ultrasonic flowmeter

0.21) to the second (washout) ventilator ($FiO_2 = 0.95$, $FiSF_6 = 0.05$) at the end of the last expiration before the washout. An ultrasonic flowmeter (Spiroson, Ecomedics, Dürnten, Switzerland) was directly connected to the endotracheal tube without any airway filter and was maintained in situ for the whole experiment. The same flow sensor was used for N_2 and SF_6 washout. The flow meter has a flow range of ± 1500 ml/s and a dead space of 3.5 ml. FRC of the nitrogen-washout (FRC_{MCN2} and FRC_{MBNW}) was calculated using the nitrogen signal of a respiratory mass spectrometer (MGA 3000, Morgan Medical, Biggin Hill, UK). FRC_{MCN2} was determined by the mixing chamber nitrogen washout technique [4, 5] utilising a computer-based data acquisition system (2600, SensorMedics Anaheim, CA). The N_2 signal for FRC_{MBNW} was acquired with the integrated AD-board of the Spiroson unit. Exhaled nitrogen volume was calculated by integrating the measured flow and by multiplying the instantaneous nitrogen fraction with the corresponding exhaled volume. FRC_{MBNW} was then obtained by dividing this exhaled nitrogen volume with the nitrogen concentration of the breathing gas prior to the washout. Corrections were made for changes in gas viscosity and delay time of the mass spectrometry signal [10, 12]. Dedicated software written in LabView 5.0 was used for analysis. Equipment dead space was subtracted for all FRC measurements techniques.

Principles of ultrasonic flow meter

Flow and molar mass of the inhaled and exhaled gas were measured with the ultrasonic flow meter [13]. Technique and mathematical models for ultrasonic flow and gas density measurement have been described previously [6, 7, 13] and are available as electronic supplementary material.

Table 1 Functional residual capacities measured in six monkeys comparing the mixing chamber technique and the molar mass method

Number =6	FRC _{M_{CN2}} (ml)	CV (%)	FRC _{M_{MM}} (ml)	CV (%)	<i>p</i> value	Difference (%)
Spontaneous breathing	139±65*	7.3	132±62*	9.2	0.02	5.0
Mechanical ventilation						
0 cmH ₂ O PEEP	252±107	2.0	256±85	4.0	0.46	1.6
5 cmH ₂ O PEEP	401±67	8.1	414±52	7.0	0.09	3.2
10 cmH ₂ O PEEP	555±51*	1.3	584±48*	5.5	0.03	5.2

Values expressed as means ± SD

FRC_{M_{CN2}} functional residual capacity measured with the bias flow nitrogen washout technique using a mixing chamber, FRC_{M_{MM}} multi-

ple breath nitrogen washout technique using the molar mass method, CV coefficient of variance, PEEP positive end-expiratory pressure
*significantly different from FRC_{M_{CN2}}

In the present study we used 5% SF₆ and 95% oxygen as the washout gas rather than 100% oxygen, because 5% SF₆ results in a greater change of molar mass than using pure oxygen alone (changes in molar mass are 7.3 and 3.15 g/mol, respectively), and the gas mixture has a higher signal-to-noise ratio. Using the MM, the difference between the instantaneous inspiratory SF₆-O₂ fraction and the SF₆-O₂ fraction at the end of the wash-in was obtained and the SF₆-O₂ flow was calculated as the product of instantaneous airflow and the difference. The SF₆-O₂ flow was then integrated over time to give the inspired SF₆-O₂ volume. The FRC of the SF₆-O₂ wash-in was obtained by dividing the measured inspired SF₆-O₂ volume with the end tidal SF₆-O₂ concentration of the SF₆-O₂ at the end of the wash-in.

All signal data were acquired at a sampling rate of 200 Hz. Analysis of the data was done offline using adapted software.

Measurement protocol

Two different sets of measurements were performed. (1) in six rhesus monkeys, three measurements each of FRC_{M_{CN2}}, FRC_{M_{BNW}} and FRC_{M_{MM}} were measured during spontaneous breathing via the endotracheal tube. (2) After starting mechanical ventilation, FRC_{M_{CN2}}, FRC_{M_{BNW}} and FRC_{M_{MM}} were measured in six monkeys at three different levels of positive end-expiratory pressure (0 cmH₂O, 5 cmH₂O and at 10 cmH₂O). The measurements were repeated at least three times at each level. The nitrogen signal of the respiratory mass spectrometer could only be used either for FRC_{M_{CN2}} or FRC_{M_{BNW}} and, therefore, only one of each could be compared to FRC_{M_{MM}} simultaneously.

Statistics

Values are presented as means ± SD. Reproducibility of the methods was evaluated by calculating the coefficient of variation. The accuracy of the measurements (limits of agreement) between the different FRC measurement methods were assessed with the difference of the measured volumes plotted against their mean [14]. Because of the relatively small sample size in each group a Wilcoxon signed rank test was used to compare paired FRC values at different PEEP levels. A *p* value of less than 0.05 was considered significant.

Results

Three measurements in each of the six monkeys were performed during spontaneous breathing via the endotracheal tube and FRC_{M_{CN2}} and FRC_{M_{MM}} were calculated

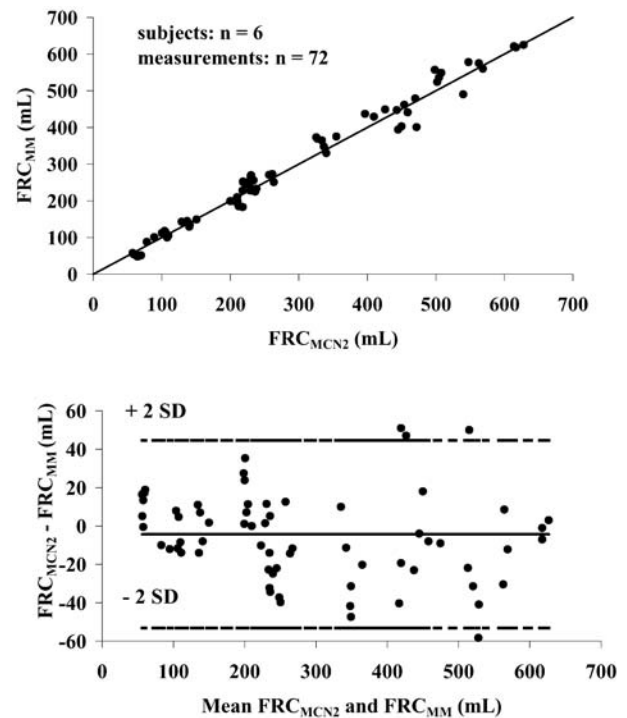


Fig. 2 Functional residual capacity (FRC) measured with the nitrogen washout using the mixing chamber (FRC_{M_{CN2}}) compared to the molar mass method (FRC_{M_{MM}}). The solid line indicates the mean of the differences and the interrupted lines the 2 SD from the mean

(Table 1). FRC_{M_{MM}} was statistically significantly lower than FRC_{M_{CN2}} but the measured difference of 7 ml (less than 5%) is clinically not relevant. In Table 1 all measured FRC_{M_{CN2}} and FRC_{M_{MM}} during mechanical ventilation at 0, 5 and 10 cmH₂O PEEP are given. Some of these measured FRC values were significantly different, but again the differences are clinically not relevant (<5%).

Figure 2 displays, in the upper panel, the relationship between all FRC_{M_{CN2}} and FRC_{M_{MM}} (measured during spontaneous breathing and mechanical ventilation) and, in the lower panel, the mean difference of the two measurements against their mean. The mean offset of all

Table 2 Functional residual capacities measured in six monkeys comparing the multiple breath nitrogen washout to the molar mass method

Number =6	FRC _{MBNW} (ml)	CV (%)	FRC _{MM} (ml)	CV (%)	<i>p</i> value	Difference (%)
Spontaneous breathing	140±50	3.7	141±54	4.6	0.3	0.7
Mechanical ventilation						
0 cmH ₂ O PEEP	212±68*	7.0	232±73*	3.8	<0.01	9.4
5 cmH ₂ O PEEP	356±63	6.3	373±57	5.6	0.27	4.8
10 cmH ₂ O PEEP	563±24	3.1	574±30	3.0	0.18	2.0

Values expressed as means ± SD

FRC_{MBNW} functional residual capacity measured with the multiple breath nitrogen washout technique using a respiratory mass spectrometer, FRC_{MM} multiple breath nitrogen washout technique us-

ing the molar mass method, CV coefficient of variance, PEEP positive end-expiratory pressure

*significantly different from FRC_{MCN2}

measured FRC_{MCN2} and FRC_{MM} was -4.3 ± 24.4 ml ($p=0.3$). FRC_{MBNW} measured during spontaneous breathing was equal to FRC_{MM} (Table 2). Table 2 lists all measured FRC_{MBNW} and FRC_{MM} at 0, 5 and 10 cmH₂O PEEP.

Figure 3 displays, in the upper panel, all measured FRC_{MBNW} and FRC_{MM} (measured during spontaneous breathing and mechanical ventilation) and, in the lower panel, the mean difference of FRC_{MBNW} and FRC_{MM} against their mean. The mean offset of all measured FRC_{MBNW} and FRC_{MM} was 10.0 ± 14.8 ml ($p<0.01$).

Discussion

The concentration of an inert gas such as SF₆ can be estimated from the molar mass signal (MM) of an ultrasonic flow meter and, thus, FRC can be calculated during an inert gas wash-in or washout. In the present study we demonstrated that, in mechanically ventilated monkeys, the inert gas wash-in technique using the MM is not significantly different to the “gold standard”, the nitrogen washout technique using a mixing chamber [4] or the multiple breath nitrogen washout technique [12, 15]. All three methods have similar intra-individual variations and detect changes of FRC accurately when PEEP is changed from 0 to 5 and 10 cmH₂O.

All three methods have their limitations. The mixing chamber bias flow technique is highly accurate [4, 5] and well established to measure lung volumes [16], but needs bulky equipment and is limited to subjects with an oxygen requirement of FiO₂ less than 0.7. FRC is calculated from the area under the curve of the nitrogen signal measured with a respiratory mass spectrometer sampling from the output of a mixing chamber. The nitrogen signal has a pyramidal shape with a slightly prolonged tail at the end of the washout. In the case of severe maldistribution of ventilation, this tail becomes more prolonged and the signal-to-noise ratio is low, which leads to difficulties in calculating FRC accurately.

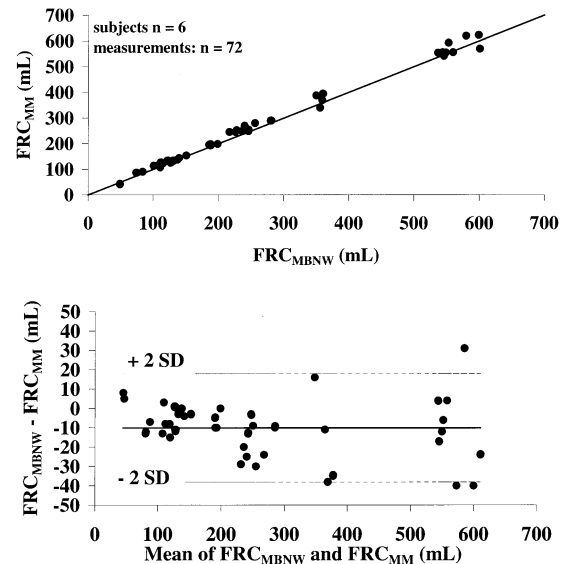


Fig. 3 Functional residual capacity (FRC) measured with the multiple breath nitrogen washout using (FRC_{MBNW}) compared to the molar mass method (FRC_{MM}). The solid line indicates the mean of the differences and the interrupted lines the 2 SD from the mean

The multiple breath nitrogen washout technique during mechanical ventilation needs two ventilators and is therefore, like the mixing chamber technique, not suitable for daily routine bedside FRC measurement. The nitrogen concentration is measured at the airway opening and can be displayed breath-by-breath on the computer screen. The nitrogen signal of the respiratory mass spectrometer has a variable delay time to the flow signal. Therefore the flow and nitrogen signals need to be aligned carefully [10, 12, 15]. Pressure changes within the ventilator circuit considerably shorten or prolong the delay time of the nitrogen signal, which makes good alignment of the nitrogen and the flow signals difficult. Any nitrogen washout technique using oxygen as a washout gas has the potential disadvantage that the

washout gas participates in gas exchange. This effect is negligible in healthy subjects and during positive pressure ventilation, but in spontaneously breathing infants it has been shown that pure oxygen rebreathing changes breathing pattern and creates atelectasis [15].

With the ultrasonic flow meter a new method is introduced to measure flow, volume and gas concentration all in one sensor. In previous studies we have shown that the tracer gas (SF_6) can be fed into the circuit of the ventilator through a side port and thus only one ventilator is necessary for FRC measurement [6, 7, 8, 9]. In the present study, however, we used an experimental set-up with two ventilators in order to compare simultaneously the nitrogen washout and the molar mass technique. FRC_{MM} measurements were on average slightly higher than $\text{FRC}_{\text{MCN}_2}$ or FRC_{MBNW} , but these differences are clinically not relevant and may be partly explained by the fact that SF_6 does not interfere with gas exchange. The reproducibility of all three methods were within the same range.

The user of the ultrasonic flow meter has to be aware of several important methodological issues. The ultrasonic flow meter has a low dead space (3.5 ml) and provides an all-in-one sensor for the flow and gas concentration (by molar mass) measurements. In order to compute the molar mass, the temperature along the sound transmission path is computed using a combination of a mathematical model and temperature measurements. If the measurements are performed during mechanical ventilation using humidified and heated air (BTPS conditions), the corrections of the MM are negligible. However, if measured under ATPS conditions (e.g. as with spontaneous breathing in room air) the temperature corrections have considerable effects on flow measurements. The MM needs to be further corrected for the wash-in/washout process occurring in the two side chambers. A step-response function assuming exponential gas mixing in the side chamber was used. This correction depends on the length of the diffusion path within the side chamber and the effusion constant α of the tracer gas.

$$\alpha = \frac{1}{\sqrt{M}}$$

In ventilated patients, the molar mass of the inhaled and exhaled gas is not constant. The molar mass of exhaled CO_2 is 36 g/mol, of oxygen 32 g/mol and of nitrogen 28 g/mol. The effusion constants of these gases are different. We therefore calculated the FRC based on the change of molar mass (delta MM) in respect to the baseline value prior to the SF_6 wash-in/washout and not in absolute terms. Using the delta MM, an almost perfect matching can be obtained if the MM is superimposed onto the SF_6 signal of the respiratory mass spectrometer (Fig. 4). The ultrasonic flow meter has not been validated under more extreme conditions such as rapid changes

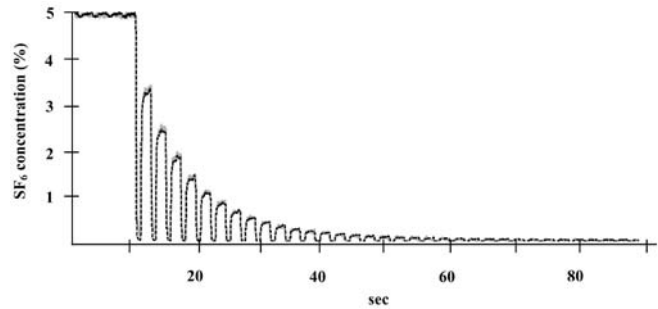


Fig. 4 The respiratory mass spectrometer signal measuring the SF_6 concentration is superimposed on the molar mass signal. Both signals were measured simultaneously. The black signal represents the molar mass signal, which has been converted to % concentration, the grey signal is the SF_6 signal of the respiratory mass spectrometer

in breathing pattern, high respiratory rate and high ventilation pressures. In a previous work [6] we showed that the molar mass, but not the flow signal, of the ultrasonic flowmeter is pressure sensitive. Using delta MM, however, the effect of pressure changes on the MM is eliminated. The ultrasonic measurement technique presented has only been validated in a small volume range of 50–650 ml and therefore cannot be directly generalised to adult humans with lung disease.

In conclusion, we have demonstrated that lung volumes (FRC) can be accurately measured with an ultrasonic flow meter if compared to standard methods such as the bias flow nitrogen washout using a mixing chamber or the multiple breath nitrogen washout. The ultrasonic flowmeter may be a clinically very helpful tool to adjust PEEP for optimal ventilation at normal FRC levels and may be valuable for more precise interpretation of pressure-volume loops. The equipment to measure FRC with the ultrasonic flow meter is simple, it requires only a small laptop computer, the switching valve and a small cylinder containing pure SF_6 . In addition, breath-by-breath systems provide the potential advantage of assessing gas distribution and ventilatory efficiency [6, 7, 9, 15] that cannot be measured by the bias flow washout systems.

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