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Habilitationsschrift

Monitoring of lung volume in ventilated newborn infants using Heptafluoropropane as tracer gas

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„Ich kann freilich nicht sagen, ob es besser werden wird wenn es anders wird; aber so viel kann ich sagen, es muss anders werden, wenn es besser werden soll.“

GEORG CHRISTOPH LICHTENBERG

List of abbrevations

CDH	congenital diaphragmatic hernia
CO_2	carbon dioxide
CT	computed tomography
CV	coefficient of variation
FRC	functional residual capacity
FRC_{CT}	functional residual capacity measured by computed tomography
FRC_{HFP}	functional residual capacity measured by heptafluoropropane multiple breath washout
HFP	heptafluoropropane
LCI	lung clearance index
M_1/M_0	first to zeroth moment ratio
M_2/M_0	second to zeroth moment ratio
MBW	multiple breath washout
mL	milliliter
PEEP	positive end expiratory pressure
PIP	positive inspiratory pressure
SF_6	sulfur hexafluoride
V_D	dead space
VI	ventilation inhomogeneity
V_T	tidal volume
V_TBL	tidal volume measured by the Draeger BL 8000 ventilator
V_TCT	tidal volume measured by computed tomography

1. Introduction

1.1. Perinatal morbidity

Perinatal mortality is one of the most sensitive indices of healthcare both for the mother during pregnancy and to the newborn infant. In 2010, 667947 newborn infants were born alive in Germany [1]. In Berlin nearly 18% of all newborn infants were admitted to a pediatric ward [2], and 6.7% of newborns were classified as premature (gestational age less than 37 postmenstrual weeks). Improvement in prenatal, obstetric, pre- and neonatal care over the last three decades has lead to dramatic reduction of infant mortality rate, which at present has reached a value close to 4‰ (see fig. 1) [1].

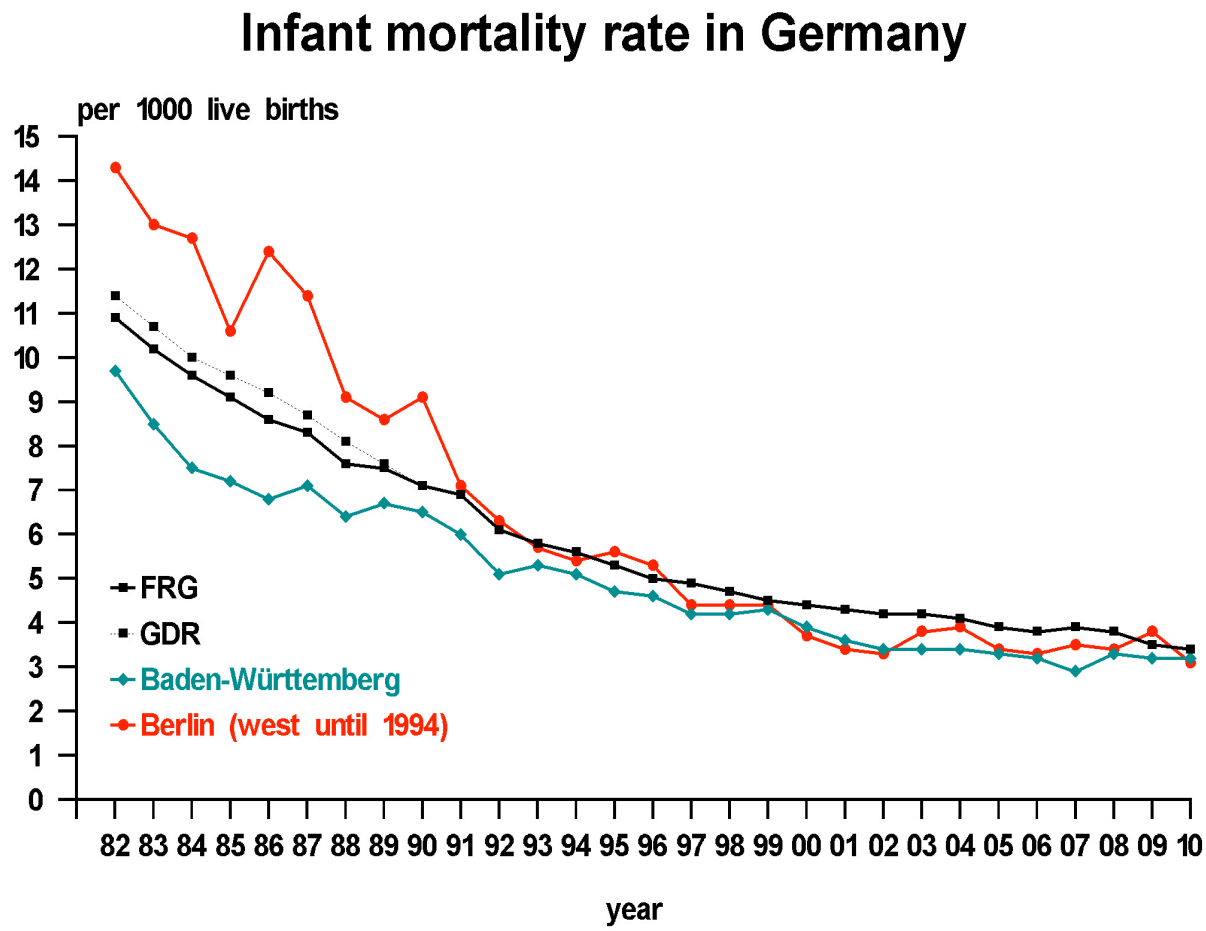

Fig. 1: Infant mortality rate in Germany. Depicted are FRG, GDR, Baden-Württemberg and Berlin over the years 1982 until 2010, adapted from [1, 2]

1.1.1. Respiratory morbidity

The German neonatal quality assurance dataset and the national birth/death registry differ considerably and deaths in extremely low birthweight infants may be missed [3]. Despite this potential bias, there is consensus about the high percentage of respiratory morbidity. [4] This has lead to specific and targeted respiratory support in neonatal tratment since more than 50 years [5-7]. Respiratory therapy, however, has to address two essential reasons in postnatal care: immaturity and pulmonary diseases [8].

1.2. Treating the respiratory distressed newborn infant

Beside prenatal lung maturation and postnatal surfactant administration, one of the most common therapeutic interventions targeting respiratory insufficiency in neonatal medicine, consists in using respiratory assisting or ventilating systems [9, 10].

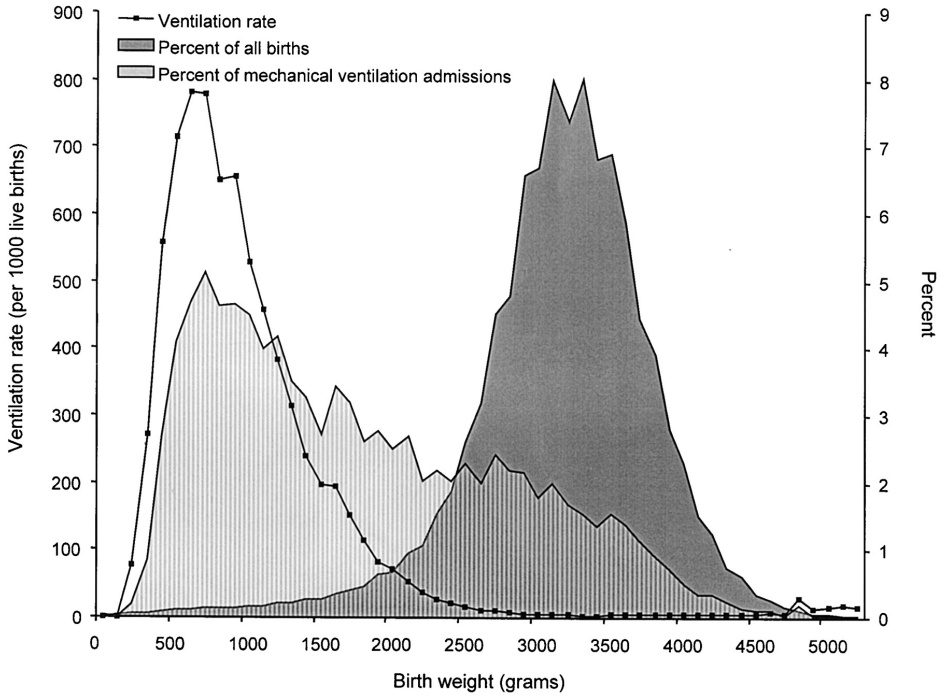

Fig. 2: Incidence of ventilation rates and percentage of mechanical ventilation admissions depending on birth weight (see lit. [9])

To prevent ventilator-induced lung injury, noninvasive ventilation is meanwhile preferred by neonatologists, but invasive ventilation is still necessary for support of neonates with insufficient spontaneous breathing. The use of a respirator to ventilate newborn infants is reflected by low birth weight (immaturity) and prevalent existing pulmonary diseases [8]. The incidences of ventilation rates are depicted in fig. 2.

1.2.1. Volume in neonatal ventilation

The frequent therapeutic application of ventilatory support in neonatology resulted in the design and development of different neonatal ventilators; however, primary parameters to set were ventilatory pressures and times. In 1954, tidal volume (V_T) was integrated for the first time as a steering factor for ventilation into a neonatal ventilator [11]. By 1960 Abramson´s book [12] listed 21 neonatal ventilators produced in USA. Meanwhile, due to further technical development, vitually all neonatal ventilators feature diverse modes of ventilation using V_T, be it as measured parameter or as a control factor. Actual systematic reviews and meta-analyses point out that the implementation of volume control and volume-targeted ventilation in neonatology is superior to pressure-limeted ventilation. Volume-targeted ventilation was beneficial in terms of reduced volu- / baro-trauma and reduced death / bronchopulmonary dysplasia [13]. Furthermore, superiority regarding decreased work of breathing and respiratory acidoses was evident [13, 14]. The application of volume-targeted, compared with pressure-limeted ventilation reduced the duration of ventilation and the incidence of severe intraventricular hemorrhages [15]. These improvements in neonatal outcome lead to the spread of volume control and volume-target in neonatal ventilation [16].

1.2.2. Functional residual capacity and postnatal adaptation

Increasingly the amount of air remaining in the lung at the end of passive expiration, called functional residual capaity (FRC) has become a focus of intense research [17-20]. The FRC limits the fluctuation of alveolar gas concentrations within each breathing cycle, serves as an oxygen buffer and equals the lung volume in resting expiratory position under normal conditions [20, 21]. To overcome the elastic resistance the resting expiratory position is ideal due to the lowest forces, necessary [22]. Actual studies of neonatal adaptation showed that formation and stabilisation of the FRC are crucial for physiological adaptation [23-25]. However, in neonatology, formation and stabilisation of the FRC do play a role beyond postnatal adaptation. The FRC as well is of considerable interest in conjunction with the application of surfactant [26] and during severe respiratory failure [27].

A close correlation exists between alveolar gas exchange and pre- und postnatal development of the lung [21, 28]. Compared to adults the lungs of newborn infants are characterized by an instable and lower FRC [29, 30], lower absolute pulmonary compliance and higher absolute pulmonary resistance [31]. In addition, the postnatal switch of the

circulation frequently involves higher pulmonary resistance, leading to right-to-left shunting via open fetal conjunctions (foramen ovale, ductus arteriosus Botalli) [32]. During this phase of transition in particular, the FRC allows for a normal and stable gas exchange [25]. The FRC depends on both elastic (bias of volume reduction) and chest wall (bias of volume expansion) forces of the lung [22, 33]. Thus, changes in this relationship may have an impact on the FRC and the closing volume, which is of particular interest in case of ventilatory support by mechanical ventilation. Already 50 years ago Auld et. al demonstrated by body plethysmography that respiratory distress in newborn infants was associated with lower thoracic gas volume [30]. However, these measurements were carried out in spontaneous breathing infants [34-36].

1.2.3. Measuring functional residual capacity in neonatology

Currently, FRC can be measured by two major techniques: plethysmography (utilizing infant body-boxes), and gas dilution or washout techniques. Bodyplethysmography measures the total gas in the thoracic cage (and the small amount in the abdominal and oral cavities), irrespective of whether the gas is freely communicating or trapped behind obstructed airways. The gas dilution techniques can measure only that gas which is freely communicating. In the early sixties body plethysmographic measurement of thoracic gas volume in newborn infants was described first [29, 30]. Thoracic gas volume equals FRC in normal subjects, the technique being derived from duBois et al.[37] In the past the most commonly used dilution or washout techniques are helium dilution (a closed-circuit method) [34-36, 38], and nitrogen washout (an open-circuit method) [39, 40]. With the open circuit device tidal flows and nitrogen concentrations are analyzed either for a few consecutive breaths, or until complete washout has occurred [41-43]. Meanwhile, other gases can be employed, of which sulphur hexafluoride (SF_6) is most commonly employed [44-46]. While the helium dilution method, besides potential difficulties as air leakages and CO_2 accumulation, has the disadvantage of adding compliance to the respiratory system and does alter positive inspiratory pressures [47]. Bodyplethysmography, however, needs special adjustments and is too cumbersome for bedside measurements of ventilated newborn infants [48].

The method for measuring FRC that has gained most acceptance in newborns and infants is based on the technique of Gerhardt et al. [40, 49, 50] and has been validated in several studies [40, 50-53], however, best fitted in spontaneous breathing newborn infants [54, 55].

1.2.4. Functional residual capacity in ventilated patients

The knowledge of the alteration of FRC, a function of lung size, airway obstruction, restriction, and mechanical properties of the lung would be helpful for both monitoring and steering mechanical ventilation. Rapid changes in lung volume due to different diseases [56-58] and the effect of the appropriate treatment [59-61] otherwise might be missed. Furthermore, the use of medications eg. anaesthetic volatile gases [62], anaesthetics [63] and neuromuscular blockade [64] destabilises FRC further and may thus impair oxygenation [32]. Thus, it is obvious that measurement of FRC under clinical conditions is desired. However, so far a simple application of FRC measurement in ventilated newborn infants is not achieved because the existing devices are too cumbersome [43, 49, 50, 65] and have not been automated commercially. Furthermore, the addition of compliance and resistance to the ventilation circuit [66], as well as oxygen interference in patients with severe lung disease requiring markedly elevated inspired oxygen concentrations may lead to measurement errors in both the helium dilution and the nitrogen washout method [67, 68]. The use of SF_6 as tracer gas in ventilated newborn infants [46] is, at least in Germany subject to scientific questioning because up to now it is not registered as medical gas. Furthermore, its use requires, unlike V_T measurements and blood gas sampling, parental consent [69]. Further problems arise from the poor pressure stability of the gas sensors [18, 69] and the potential impact of leakage [70, 71], which is common in neonates due to the use of uncuffed tubes.

1.3. Aims of the thesis

Results of FRC measurements by tracer gas multiple breath washout (MBW) in ventilated adults or toddlers cannot be adopted without objections to ventilated newborn infants. Aiming to both develop a feasible and determine the predictive value of a clinically applicable and easy to handle FRC measurement tool for ventilated neonates, we sought to investigate the following questions:

1. Is the inert and certified medical gas „1,1,1,2,3,3,3-heptafluoropropane (HFP)“ a suitable tracer gas for MBW technique?
2. Can reproducible measurements be performed in ventilated small lungs using HFP and is the sensitivity sufficiently high to demonstrate changes in the functional residual capacity and ventilation homogeneity?
3. Does dynamic multislice computed tomography allow for calculation of FRC comparable to MBW technique using HFP?
4. Are volumes of small lungs, measured by HFP MBW, affected by changes in ventilator settings and may this be a possible pitfall for misinterpretation?
5. Has the body position of small individuals (prone vs. supine) during ventilation an effect on lung volumes, measured by MBW method using HFP?
6. Is the HFP MBW technique suitable for measuring the effect of surgery on the lung under clinical conditions and does it represent a useful tool for monitoring ventilated newborn infants?

2. Results

2.1. Investigating heptafluoropropane (HFP) as tracer gas for lung function measurements in a newborn piglet model

Auwaerter V, **Proquitté H**, Schmalisch G, Wauer RR, Pragst F. Determination of 1,1,1,2,3,3,3-Heptafluoropropane (HFP) in Blood by Headspace Gas Chromatography-Mass Spectrometry. J Anal Toxicol. 2005; 29 (6): 574-6

To investigate the first question regarding the inert and certified medical gas 1,1,1,2,3,3,3-heptafluoropropane (HFP), newborn piglets were investigated. A sensitive method for quantitative determination of HFP in blood was developed using headspace gas chromatography–mass spectrometry with nitrogen as internal standard. Briefly, newborn piglets were ventilated for 30 min with air containing 0.8% HFP (v/v). Blood samples were taken and HFP was measured, the limits of detection and quantification being 0.005 and 0.016 mg/L, respectively. A saturation concentration in blood close to 2.5 mg/L was attained after 3 min ventilation and decreased within less than 5 min after termination the HFP supply. Furthermore, no relevant accumulation of HFP during measurement of functional residual lung capacity by MBW techniques was observed.

2.2. Appraisal of reproducibility and accuracy of HFP MBW both in a lung model and ventilated newborn piglets

Proquitté H, Kusztrich A, Auwaerter V, Pragst F, Wauer RR, Schmalisch G. Functional residual capacity measurement by heptafluoropropane in ventilated newborn lungs: In vitro and in vivo validation. Crit Care Med. 2006; 6 (34): 1789-95

In this study we could prove that HFP can be detected in low concentrations after inhalation (0.8%) without relevant accumulation over 30 minutes. Thus, its use as a tracer gas seemed feasible to measure FRC and ventilation homogeneity by MBW during mechanical ventilation. To study accuracy and precision of the measurement a lung model (a syringe and a bellow with a volume range from 11 to 35 mL) was introduced. Furthermore, sixteen newborn piglets (median weight 1390 g) were prospectively investigated before and after surfactant depletion (PaO_2 <100 torr in FiO_2 = 1.0). A new infrared mainstream sensor connected with the flow sensor of the ventilator (Draeger Babylog 8000) served to measure HFP concentrations. Reproducibility of the method and its sensitivity to detect changes of FRC were assessed in vivo by variation of ventilatory variables. The absolute error of FRC in vitro was <1mL (relative errors <3%) with a coefficient of variation (CV) <4%. In vivo CV of consecutive measurements was only slightly higher (<5.1%). In healthy piglets, the increase in peak inflation pressure (PIP) and positive end-expiratory pressure (PEEP) by 3-4 cm H_2O lead to a moderate rise in FRC (20.9 +/- 8.6 vs. 26.0 +/- 11.9 mL/kg, p=0.17) but had no effect on ventilatory homogeneity. Lung lavage itself, despite increased ventilatory pressures, reduced FRC significantly to 14.5 mL/kg ($p<0.05$) while both lung clearance index (LCI) ($p<0.001$) and moment ratios ($p<0.01$) increased significantly due to uneven alveolar ventilation. However, after surfactant-depletion, the additional elevation of ventilatory pressures compared to healthy piglets now accounted for a significant increase in FRC (14.5 +/- 6.7 mL/kg to 29.9 +/- 12.6 mL/kg; $p<0.001$) whereas lung clearance index (LCI) and moment ratios decreased ($p<0.01$). Thus we concluded that HFP is a suitable tracer gas for precise FRC measurements tested in vitro and allows for reproducible measurements in ventilated small individuals. The sensitivity of the MBW method using HFP was sufficiently high to demonstrate the effect of changes in ventilatory settings on FRC and ventilation homogeneity. Again, comparable with the first study, HFP concentrations in blood showed no significant accumulation for repeated FRC measurements.

2.3. Allows dynamic multislice computed tomography for calculation of functional residual capacity (FRC) comparable to MBW technique using HFP in ventilated newborn piglets

Elgeti T, **Proquitté H**, Rogalla NE, Mews J, Wauer RR, Hamm B, Schmalisch G, Rogalla P. Dynamic computed tomography of the neonatal lung: volume calculations and validation in an animal model. Invest Radiol. 2005; 40 (12): 761-5

In newborn piglets we sought to validate dynamic lung volume calculation by respiratory-gated multislice computed tomography (CT) compared with HFP MBW. Mechanically ventilated newborn piglets were imaged in a multislice CT with 0.5-mm slice thickness (4:16 pitch, 0.5-second rotation time, 120 kV). For recording the respiratory signal the respirator (Draeger BL 8000) was connected to the CT unit. V_T and FRC was measured by the respirator and by using the previously described HFP multiple-breath washin-washout technique, respectively. All animals were scanned in 3 different ventilator settings. Mean FRC determined by CT did not differ from washout technique 24.7+/-8.6 mL vs. 24.8+/-7.3 mL (P=0.555). Pearson's correlation coefficient, however, showed a strong correlation between the data obtained with CT and that obtained with MBW (r=0.886). A similar correlation (r=0.837) without significant differences in the mean tidal volumes (CT: 8.9+/-2.4mL and BL 8000: 8.7+/-2.4mL, P=0.566) was achieved after exclusion of one outlier. Thus, FRC values measured by HFP MBW could be convincingly confirmed by CT.

2.4. Critical assessment of volume measurement by HFP MBW and its affection by changes in ventilator settings

Schmalisch G, **Proquitté H**, Roehr CC, Wauer RR. The effect of changing ventilator settings on indices of ventilation inhomogeneity in small ventilated lungs. BMC Pulm Med. 2006; 18; 6: 20

Using MBW techniques in ventilated newborns for measuring both lung volume and ventilation inhomogeneity (VI) is impeded by high accessory apparatus dead space. We investigated by modelling and by in-vivo measurements the impact of changing ventilator settings on VI indices in small lungs. The homogeneity of the alveolar ventilation was assessed by LCI and the moment ratios of the HFP MBW curve. The modelling showed a strong connection of both the LCI and the moment ratios to the tidal volume (V_T), dead space (V_D) and the FRC. Resulting computed findings were compared with measured effects in 15 ventilated newborn piglets (median weight 1135g) in whom PIP had been increased. Increased PIP lead to a significant rise in both V_T (15.4 +/- 9.5 vs. 21.9 +/- 14.7; p=0.003) and FRC (31.6 +/- 14.7mL to 35.0 +/- 15.9mL; p=0.006), whereas LCI (9.15 +/- 0.75 vs. 8.55 +/- 0.74; p=0.019) and the moment ratios (p<0.02) decreased significantly. Within-subject variability of the VI indices was lower in FRC measurements (5.6%) compared to LCI (9.8%) and moment ratios (6.6% to 16.3%). Computer simulations ascertained that changes in V_T and FRC and not improvement of the homogeneity of alveolar ventilation accounted for significant changes in VI indices. Thus, in small ventilated lungs with a high dead space fraction, indices of VI measured by HFP MBW may be misinterpreted if the changes in ventilator settings are not considered.

2.5. Investigating the positioning of newborn piglets during ventilation as a possible effect on lung volume measurement by HFP MBW

Proquitté H, Elgeti T, Roehr CC, Rogalla P, Wauer RR, Schmalisch G. Comparison of lung volume measurements by multiple breath heptafluoropropane washout and computed tomography in small ventilated lungs. Med Sci Monit. 2009; 15 (10): 275-80

Knowledge of lung volumes is essential for monitoring and optimization of mechanical ventilation. Using the model of ventilated newborn piglets, HFP MBW and respiratory gated computed tomography (CT) were performed simultaneously to determine the effect of positioning (supine and prone position) on lung volume measurements with either method. In 6 ventilated piglets (median weight 945g) blood gases, respiratory mechanics and lung volumes were measured in randomly order both in prone and supine position. FRC was measured simultaneously by HFP MBW (FRC_{HFP}) using the previously described infrared mainstream sensor and by CT (FRC_{CT}) at the end of inspiration and expiration. Tidal volume (V_T) was measured both by the Dräger Babylog 8000 ventilator (V_TBL) and by the volume difference of the CT scans (V_TCT). We found a strong correlation (r=0.97) between FRC_{HFP} (25.2±8.5mL) and FRC_{CT} (24.9±7.6mL) without significant bias. Nevertheless, differences in Bland-Altman limits of agreement varied between –19.7% and 19.5% indicating discrapencies between both volume measurement techniques. Analogous FRC measurement, a strong correlation (r=0.91) between V_TBL (8.5±2.0mL) and V_TCT (9.0±2.4mL) was ascertained without statistically significant bias, the limits of agreement fluctuating between –24.4% and 14.0%. We could show that body position (prone vs. supine), however, had no significant effect on lung volumes measured by either technique, indicating that HFP MBW may be feasible for clinical use.

2.6. Varifying the HFP MBW method under clinical conditions in ventilated newborn infants requiring postnatal surgery

Proquitté H, Freiberger O, Yilmaz S, Bamberg C, Degenhardt P, Roehr CC, Wauer RR, Schmalisch G. The effect of surgery on lung volume and conventional monitoring parameters in ventilated newborn infants. Eur Respir J. 2010; 35 (5): 1072-8

In a clinical study, we sought to investigate the afore described HFP multiple breath washout method in newborn infants in the clinical setting. Immediately postnatally, thoraco-abdominal surgery is a serious intervention with respect to gas exchange and lung mechanics. Therefore we prospectively compared surgery-induced changes in FRC and VI indices with changes in conventional monitoring parameters. 29 ventilated newborns (mean weight 2,770+/-864 g at surgery) were investigated, of whom 13, 9 and 7 underwent thoracic, abdominal or congenital diaphragmatic hernia (CDH) surgery, respectively. Measurements were performed <6 h before surgery, 22-24 h after surgery and <6 h before extubation, therby recording gas exchange, respiratory mechanics, FRC and VI index data. Surprisingly, thoraco-abdominal surgery resulted in changes to FRC and VI indices in a procedure-specific manner. While FRC decreased in non-CDH infants, FRC increased and VI indices decreased in CDH infants. However, neither in conventional mechanical nor ventilatory monitoring parameters these changes were reflected. Furthermore, despite improvements, the differences in FRC and VI between CDH and non-CDH infants indicated persistent impaired lung function in CHD infants. This enduring impairment only was distinguishable by implementing HFP MBW because FRC and VI indices changed following surgery, conventional monitoring parameters, however, did not.

3. Discussion

In the studies contributing to this thesis, we considered different aspects of a feasible and easy to handle tool for lung volume measurements for ventilated neonates during its development from bench to bedside. In contrast to the widly used SF_6, HFP is a certified medical gas; however, its use for FRC measurement in newborns was not investigated up to now. Therefore the FRC measurement under mechanical ventilation was investigated at different steps. Firstly, accuracy and reproducibility of FRC measurements using the HFP MBW technique was assessed using both a mechanical lung model and newborn piglets. Secondly, the impact of changes in the ventilator settings on lung volumes, measured by HFP MBW, was evaluated. Thirdly, simultaneous lung volume measurement by HFP MBW, and by dynamic multislice CT were compared and the impact of positioning on the patient was investigated. Forthly, its applicability under clinical conditions was tested in ventilated newborn infants requiring postnatal surgery and the predictive value of HFP MBW determined.

With respect to the introduction of the chemically inert and nonflammable hydrofluoroalkane HFP [72, 73] into the setting of lung volume measurements during ventilation, some aspects have to be considered. Data on HFP, also known as apaflurane, Solkane® 227, or HFA-227ea, are available regarding its deposition [74] and drug delivery [75, 76] when used as an aerosol. However, if used as tracer gas, accumulation of HFP to a higher extent in the body needs to be excluded. Therefore, a sensitive headspace gas chromatography–mass spectrometry method was applied and the HFP concentration assessed in the blood during FRC measurements in newborn piglets [77, 78]. To the best of our knowledge, our study was the first to demonstrate that the addition of 0.8% (v/v) HFP to the breathing air over 30 min increased the HFP concentration in blood within 3 min and attained a maximum value close to 2500 µg/L after 10 min. Furthermore, disconnection of HFP supply to the breathing air has lead to a rapid decrease to 500 µg/L within 3 min, resulting in a half life calculation close to 0.5 min. Thus, no relevant accumulation of HFP occured in the blood, neither in healthy, nor in surfactant depleted piglets [78]. The HFP concentrations found after the FRC measurements only ranged from <5 to 85 µg/L and therefore stayed far below the saturation concentrations found by Auwärter et al [77]. Comparable results for other tracer gases are not available. This is of specific interest, as interactions with surfactant, known at present for both SF_6 [79-81] and HFP [82-85] may partly affect uptake. Therfore, interaction

with surfactant could be a limitation if either hydrofluoroalkane is used as tracer gas preferentially in newborn or premature infants. Furthermore, the fact that the measurement procedure during HFP tracer gas technique usually lasted less than 2 min and that all FRC measurements were finished with an HFP washout, diminished a relevant impact on uptake. Since comparable low solubilities of both HFP [86, 87] and SF_6 [88-91] exist, problems could further arise. SF_6 is associated with pulmonary edema [79, 92] and a potential toxicity [93-96], whereas cardiac sensitization is assigned to the volatile HFP [97]. Our measurements, however, did not discover any adverse effects on the monitored circulation variables. In summary HFP preferably suites as tracer gas, because it is already used for certified medical drug delivery [75, 76] and beyond that does not serve as greenhouse gas [98-101] and its high volatility allows for repeated FRC measurements within short time intervals.

Regarding the investigations both in the model and the piglets [78], we aimed to report on accuracy and reproducibility of the HFP tracer gas method. Both are of particular interest if a test assembly using a new tracer gas is implemented. The strong correlation (r=0.996) between the measured FRC and the volume of the model (syringe) added up to measuring errors and a coefficient of variation less than 3% and 4%, respectively. This is in line with previous results, applying Nitrogen [54, 55, 102] or SF_6 [45, 46, 103] as tracer gas, however, despite similar error size the coefficient of variation was lowest in our study, using HFP. The fact that the mean FRC error was 1.4%, satisfying the recommendation for FRC measurements by the European Respiratory Society / American Thoracic Society [104, 105] (<2.5% or minimal 2 mL of the infant lung volumes) further supports the use of HFP tracer gas technique.
In order to assess the reliability of our measurement in vivo, deviations in FRC between wash in and washout were considered. Values higher than 20% occurred in 6.1% and 6.6% in healthy and surfactant-depleted lungs, respectively, leading to repeated measurements, whereas the absulute deviations in FRC between wash in and washout in healthy and surfactant-depleted piglets were 1.3 mL and 0.9 mL, respectively. Published data of MBW FRC using nitrogen [106] act on the assumption that the short-term variability of the FRC is much lower and does not exceed 20% in healthy infants [107]. In our study median CVs of FRC measured during wash in and washout were 4.1% and 5.1%, respectively, demonstrating that there are no clinically relevant differences between both measurements. These results are in good agreement with FRC

measurements by SF_6 in spontaneous breathing infants, 3.7% for mass spectrometer [46] and 5.2% for ultrasonic flow meter [107], and ventilated healthy infants, 5.5% for ultrasonic flowmeter [108].

Notwithstanding, our animal studies suffer from limitations, and it will be a matter of further study to investigate whether our results can be directly applied to the clinical setting. Furthermore, the interpretation of significant changes in VI indices may be misleading if their dependency on the ventilator settings is not considered. Particularly in small lungs a relatively high V_D fraction increases the sensitivity of VI indices to parameter changes. Any changes in V_D or in V_T and FRC (e.g. by changing of ventilator settings or by surfactant substitution) will affect the VI indices and therefore hamper their comparability. As most VI indices increase with increasing V_D/V_T [109] newborns may emerge much higher VI indices [78, 110, 111] than spontaneously breathing children [103, 112, 113]. Most likely, these higher values in newborns rather express functional dependencies than refer to impaired alveolar ventilation. However, it was surprising that the VI indices measured in healthy piglets fairly agreed with the calculated VI indices of a uniformly ventilated volume [109]. Despite high VI indices indicate a more complex ventilation distribution within the lungs of the piglets, we could describe by HFP MBW [78] that this increase was exclusively caused by changed lung volumes.

In infancy the LCI, which describes the number of turnovers to lower the end tidal tracer gas concentration to 1/40th of the starting concentration, is one of most frequently used VI indices [113-117] and easy to comprehend. In theory, the LCI is a static value of the flat tail of the washout curve. It may vary, if the signal is noisy thereby explaining its increased within-subject variability. The major limitation of the LCI, however, is clearly shown in our study [109]. With increasing V_D/V_T but not V_T/FRC the LCI rises significantly. In addition, moment ratios, considering the whole tracer gas washout curve are abstract mathematical measures, reflecting either the first part (M_1/M_0) or the tail (M_2/M_0) of the washout curve [102, 109, 111, 118]. Therefore, the within-subject variability of M_2/M_0 is distinctly higher compared to M_1/M_0 and similar to the within-subject variability of the LCI. Compared to LCI measurements, demands on the washout curve for moment analysis are higher [104, 105, 119, 120]. It requires of a rapid rise of the tracer gas after the switch-on in order to reach the full tracer gas concentration during the first inspiratory cycle. The supply of the tracer gas into the inspiratory limb of the ventilator circuit, far from the endotracheal tube, may delay such a swift rise, which is tolerable for FRC

measurements but will affect the calculation of the moment ratios. This has to be considered for clinical interpretation of changing values as a result of modified ventilatory parameters. The central problem of all moment ratios, however, is their dependency on the number of evaluated breathing cycles [111, 121]. With the computer simulation we could provide data that theoretical values for M_1/M_0 and alveolar-based mean dilution numbers were not reached due to the finite number of evaluated cycles [109]. This hampers the comparability of the data between different laboratories if the start and the end of the evaluated breathing cycles are not specified [104, 105, 119]. Despite these limitations, a marked change can be observed with the availability of dead space-minimized mainstream gas analyzers. There is increasing interest in measuring VI by MBW techniques [18, 63, 64, 102, 108, 110, 112, 122, 123]. However, the use of VI indices in small ventilated lungs needs particular attention. Due to a relatively high dead space fraction [124] most indices are significantly affected by ventilator settings [78, 109] as changes in V_T and FRC, as well as changes in the apparatus dead space hamper both their comparison and validity.

Regarding our bench investigations, we managed to report on ventilated piglets that were investigated simultaneously by HFP MBW using the Draeger Babylog 8000 ventilator and by dynamic lung CT for comparison of lung volumes (FRC, V_T) measured by either technique. To the best of our knowledge, our studies [125-127] were the first to demonstrate a strong correlation both in FRC (r=0.886) and V_T (r=0.837) between both methods. This is in line with data from Chiomello et al. [128] who also showed that lung volume measurement, both with helium dilution and modified nitrogen washout technique correlated well with CT scanning. This is supported by the fact that under static conditions a significant correlation between lung volume and lung function already has been shown in adults [129, 130]. Thus, that tracer gas muliple breath washout appears to be easily used in clinical practice with sufficient accuracy. The effect of body position on lung aeration, however, is currently a matter of controversy. With both techniques used in our studies, no effect of body position on lung volume measurement was observed. In the past, Gattinoni et al. [131-133] showed by CT in ventilated adults that changing the body position redistributed atelectatic areas. Despite short-term effects, however, prone positioning was not found to positively influence patients outcome, neither in adults [134] nor in pediatric patients [135, 136]. In well agreement with our study, an effect of body position on the measured FRC was neither detectable in neonates [137, 138]

nor in preterm infants [139]. However, the influence of body position on lung volume in surfactant depleted lungs [140] cannot be excluded.

Notwithstanding, we showed a strong correlation between volume measurements by HFP washout and CT scan; however, some aspects have to be considered. Despite measured differences in this study were small and negligible from a clinical point of view, substantial differences between the two techniques cannot be neglected. Even with reduced radiation dosage [126] CT volume calculation involve a high radiation exposure. The necessary transport and its cumbersome application make volume measurements by CT uneligible for wider clinical use in mechanically ventilated infants, restricting it to specific indications. HFP MBW, on the other hand, can easily be performed at bedside both in the neonatal intensive ward and during operation and does not affect mechanical ventilation [78, 141]. Therefore it is currently the only clinically practical method for measuring FRC in ventilated infants. Furthermore, the washout curve provides additional information about VI [109, 142].

The clinical setting of ventilated newborn infants requiring postnatal surgery served to prove both applicability and predictive value of HFP MBW. Our study has shown that HFP-MBW is a suitable technique to measure the effect of surgery on the lung. The main finding was that FRC and VI indices measured during mechanical ventilation were more affected by surgery than conventional monitoring parameters. Furthermore, the effect on FRC and VI indices was procedure-specific. Prior to surgery, lung function measurements were similar for the infants requiring abdominal or thoracic surgery. CDH infants with impaired pre-natal lung development [143-145], however, had profound lower FRC and respiratory compliance and higher VI indices, indicating impaired ventilation homogeneity. The low postnatal FRC values in all groups may be possibly affected by neuromuscular blockade [64]. While FRC and VI indices improved in CDH infants [141] 24 h after surgery, FRC decreased further in non CDH infants. This is in line with few studies on respiratory mechanics measured pre- [146, 147] and post surgery [148-150] because primary surgical closure of abdominal wall defects was associated with deterioration of lung function [148, 151, 152]. The increase over time of FRC and compliance in our CDH infants, however, is similar to those reported by DINGER et al. [153]. Whether this is due to a stable PEEP [154] or the missing of routinely inserted chest tubes is open for discussion.

Before extubation, the differences in lung function measurements between CDH and non CDH infants had reduced, yet still remained. The observed faster increase in FRC

compared with respiratory compliance in CDH infants agrees with a recent own study [155] showing that CDH patients had worse tidal breathing parameters and lower respiratory compliance after discharge compared with non-CDH patients. FRC values prior to extubation were slightly lower than that previously reported in older infants using SF_6 as tracer gas [63, 64, 122]. However, the present FRC values are consistent with those for ventilated preterm infants [57, 61] and nonventilated term control infants [156]. MBW techniques are susceptible to endotracheal leakage [106, 157, 158], a common problem in ventilated newborn infants [70, 71], which cannot be easily overcome [159] but was lower than 20% in 95% of all infants studied [141].

Tracer gas MBW characteristics (e.g. V_D, gas sensors used, properties of tracer gases and number of evaluated breathing cycles during tracer gas MBW) are highly method dependent. MBW in ventilated newborn infants usually is performed using custom-made equipment and nitrogen [18, 160], helium [45, 53, 161] or SF_6 [18, 45, 46, 60, 153] as tracer gases. A commercial system for MBW in ventilated patients [18, 103] using ultrasound spirometry with SF_6 as tracer gas [63, 64, 107, 122, 162] is characterized by a high V_D and a bulky and heavy measuring head. On the contrary, our HFP measuring head was designed for use in small lungs and is smaller and lighter.

Summing up all studies presented, we provide evidence that HFP MBW can be used as a valuable tool for monitoring lung volume in ventilated newborn infants. Being more sensitive and more specific compared to conventional monitoring parameters, changes in lung volume can safely be determined during mechanical ventilation by HFP MBW. Corresponding, the effect of different treatment strategies (eg. surgery, surfactant application or medication-based treatment) could be monitored prospectively and changes in treatment accordingly be adapted. Notwithstanding, the present HFP MBW technique available, is limited due to the current V_D of the combined flow / HFP sensor, which still is high, especially for extremely premature infants. Hopefully, technical progress and increased efforts by the manufacturers will bring forward a new, lightweight mainstream sensor with a V_D <1mL for reliable measurements even in this very sensitive population.

4. Summary

This thesis contributes to the area of neonatal lung function research in ventilated newborn infans. Our studies have shown for the first time that heptafluoropropane (HFP) allows for safe, accurate, and reliable functional residual capacity (FRC) measurements and reproducible calculations of indices of ventilation homogeneity in ventilated healthy, sick and surfactant-depleted small lungs. HFP is an alternative tracer gas to the commonly used sulfur hexafluoride for the multiple breath washout (MBW) technique and its high volatility allows for repeated FRC measurements within short time periods without any influence on ventilator settings. Although a suitable reference method for FRC measurements in vivo was not available, the sensitivity of HFP MBW is sufficiently high to demonstrate even small treatment effects. By providing evidence that HFP MBW is a useful technique to assess the effect of thoraco-abdominal surgery on the lung, this new method was finally evaluated in the clinical setting. The effect of surgery on FRC and ventilatory inhomogeneity indices was procedure-specific, however, these changes were not reflected in mechanical or ventilatory variables. A prerequisite for clinical use of HFP MBW is that the measuring sensor be miniaturised so that measurements in premature infants are possible and that this technique is integrated into the standard monitoring of neonatal ventilators.

5. Literature

1. Statist. Bundesamt. Bevölkerung und Erwerbstätigkeit: Zusammenfassende Übersichten Eheschließungen, Geborene und Gestorbene. Destatis 2010.
2. Huppmann S, Metze B, Buehrer C. Ergebnisse der Berliner Neonatalerhebung im Vergleich. Berliner Ärzte 2010;6:32-4.
3. Hummler HD, Poets C. [Mortality of extremely low birthweight infants - large differences between quality assurance data and the national birth/death registry]. ZGeburtshilfeNeonatol 2011;215:10-7.
4. Hibbard JU, Wilkins I, Sun L, et al. Respiratory morbidity in late preterm births. JAMA 2010;304:419-25.
5. Obladen M. History of neonatal resuscitation. Part 1: Artificial ventilation. Neonatology 2008;94:144-9.
6. Draeger L, Blume G. Zur Geschichte des Draegerwerkes von 1889 bis 1936: Erinnerungen eines Werksmeisters. Lübeck, Draeger 1994:60.
7. Wiggin SC, Saunders P, Small GA. Resuscitation. The New England journal of medicine 1949;241:413; passim.
8. Touch SM, Shaffer TH, Greenspan JS. Managing our first breaths: a reflection on the past several decades of neonatal pulmonary therapy. Neonatal Netw 2002;21:13-20.
9. Angus DC, Linde-Zwirble WT, Clermont G, Griffin MF, Clark RH. Epidemiology of neonatal respiratory failure in the United States: projections from California and New York. Am J Respir Crit Care Med 2001;164:1154-60.
10. Zhang X, Kramer MS. Variations in Mortality and Morbidity by Gestational Age among Infants Born at Term. The Journal of pediatrics 2009;154:358-62.e1.
11. Engstrom CG. Treatment of severe cases of respiratory paralysis by the Engstrom universal respirator. Br Med J 1954;2:666-9.
12. Abramson H. Resuscitation of the newborn infant. St Louis 1060;Moosby.
13. Wheeler KI, Klingenberg C, Morley CJ, Davis PG. Volume-targeted versus pressure-limited ventilation for preterm infants: a systematic review and meta-analysis. Neonatology 2011;100:219-27.
14. Keszler M. State of the art in conventional mechanical ventilation. J Perinatol 2009;29:262-75.
15. Wheeler K, Klingenberg C, McCallion N, Morley CJ, Davis PG. Volume-targeted versus pressure-limited ventilation in the neonate. Cochrane Database Syst Rev 2010:CD003666.
16. Klingenberg C, Wheeler KI, Owen LS, Kaaresen PI, Davis PG. An international survey of volume-targeted neonatal ventilation. Arch Dis Child Fetal Neonatal Ed 2011;96:F146-8.
17. Siew ML, Wallace MJ, Kitchen MJ, et al. Inspiration regulates the rate and temporal pattern of lung liquid clearance and lung aeration at birth. J Appl Physiol 2009;106:1888-95.
18. Schibler A, Hammer J, Isler R, Buess C, Newth CJ. Measurement of lung volume in mechanically ventilated monkeys with an ultrasonic flow meter and the nitrogen washout method. Intensive Care Med 2004;30:127-32.
19. Hammer J, Numa A, Newth CJ. Total lung capacity by N2 washout from high and low lung volumes in ventilated infants and children. Am J Respir Crit Care Med 1998;158:526-31.
20. Hammer J, Newth CJ. Infant lung function testing in the intensive care unit. Intensive Care Med 1995;21:744-52.

21. Vyas H, Field D, Milner AD, Hopkin IE. Determinants of the first inspiratory volume and functional residual capacity at birth. Pediatr Pulmonol 1986;2:189-93.
22. Nicolai T, Lanteri CJ, Sly PD. Inherent coupling of elastic and dissipative behavior of the lung through a viscoelastic time constant. J Appl Physiol 1993;74:2358-64.
23. Hooper SB, Kitchen MJ, Siew ML, et al. Imaging lung aeration and lung liquid clearance at birth using phase contrast X-ray imaging. Clin Exp Pharmacol Physiol 2009;36:117-25.
24. te Pas AB, Siew M, Wallace MJ, et al. Effect of sustained inflation length on establishing functional residual capacity at birth in ventilated premature rabbits. Pediatr Res 2009;66:295-300.
25. te Pas AB, Siew M, Wallace MJ, et al. Establishing functional residual capacity at birth: the effect of sustained inflation and positive end-expiratory pressure in a preterm rabbit model. Pediatr Res 2009;65:537-41.
26. Siew ML, Te Pas AB, Wallace MJ, et al. Surfactant increases the uniformity of lung aeration at birth in ventilated preterm rabbits. Pediatr Res 2011;70:50-5.
27. Newth CJ, Stretton M, Deakers TW, Hammer J. Assessment of pulmonary function in the early phase of ARDS in pediatric patients. Pediatr Pulmonol 1997;23:169-75.
28. te Pas AB, Wong C, Kamlin CO, Dawson JA, Morley CJ, Davis PG. Breathing patterns in preterm and term infants immediately after birth. Pediatric research 2009;65:352-6.
29. Klaus M, Tooley WH, Weaver KH, Clements JA. Lung volume in the newborn infant. Pediatrics 1962;30:111-6.
30. Auld PA, Nelson NM, Cherry RB, Rudolph AJ, Smith CA. Measurement of thoracic gas volume in the newborn infant. J Clin Invest 1963;42:476-83.
31. Stocks J. The functional growth and development of the lung during the first year of life. Early human development 1977;1:285-309.
32. Berger TM, Stocker M. [Ventilation of newborns and infants]. Anaesthesist 2004;53:690-701.
33. Nicolai T, Lanteri CJ, Sly PD. Frequency dependence of elastance and resistance in ventilated children with and without the chest opened. Eur Respir J 1993;6:1340-6.
34. Berglund G, Karlberg P. Determination of the functional residual capacity in newborn infants; preliminary report. Acta Paediatr 1956;45:541-4.
35. Berglund G, Karlberg P, Lind J. Studies of the respiration and circulation during the neonatal period. Determination of the functional residual capacity in newborn infants; preliminary report. Acta Paediatr Suppl 1955;44:136-7.
36. Geubelle F, Karlberg P, Koch G, Lind J, Wallgren G, Wegelius C. [Aeration of the lung in the newborn infant]. Biol Neonat 1959;1:169-210.
37. DuBois AB, Botelho SY, Bedell GN, Marshall R, Comroe JH, Jr. A rapid plethysmographic method for measuring thoracic gas volume: a comparison with a nitrogen washout method for measuring functional residual capacity in normal subjects. J Clin Invest 1956;35:322-6.
38. Meneely GR, Kaltreider NL. The Volume of the Lung Determined by Helium Dilution. Description of the Method and Comparison with Other Procedures. J Clin Invest 1949;28:129-39.
39. Gerhardt T, Hehre D, Bancalari E, Watson H. A simple method for measuring functional residual capacity by N2 washout in small animals and newborn infants. Pediatr Res 1985;19:1165-9.
40. Sivan Y, Deakers TW, Newth CJ. An automated bedside method for measuring functional residual capacity by N2 washout in mechanically ventilated children. Pediatr Res 1990;28:446-50.

41. Richardson P, Wyman M, Jung AL. A method of estimating the functional residual capacity of infants with respiratory distress syndrome. Crit Care Med 1980;8:667-70.
42. Sjoqvist BA, Sandberg K, Hjalmarson O, Olsson T. Method for analysing multiple-breath nitrogen washouts. 1986;24:83-90.
43. Sjoqvist BA, Sandberg K, Hjalmarson O, Olsson T. Calculation of lung volume in newborn infants by means of a computer-assisted nitrogen washout method. Pediatr Res 1984;18:1160-4.
44. Larsson A, Linnarsson D, Jonmarker C, Jonson B, Larsson H, Werner O. Measurement of lung volume by sulfur hexafluoride washout during spontaneous and controlled ventilation: further development of a method. Anesthesiology 1987;67:543-50.
45. Vilstrup CT, Bjorklund LJ, Larsson A, Lachmann B, Werner O. Functional residual capacity and ventilation homogeneity in mechanically ventilated small neonates. J Appl Physiol 1992;73:276-83.
46. Schulze A, Schaller P, Topfer A, Kirpalani H. Measurement of functional residual capacity by sulfur hexafluoride in small-volume lungs during spontaneous breathing and mechanical ventilation. Pediatr Res 1994;35:494-9.
47. Ronchetti R, Stocks J, Keith I, Godfrey S. An analysis of a rebreathing method for measuring lung volume in the premature infant. Pediatr Res 1975;9:797-802.
48. Edberg KE, Sandberg K, Silberberg A, Sjoqvist BA, Ekstrom-Jodal B, Hjalmarson O. A plethysmographic method for assessment of lung function in mechanically ventilated very low birth weight infants. Pediatr Res 1991;30:501-4.
49. Gerhardt T, Hehre D, Bancalari E, Watson H. A simple method for measuring functional residual capacity by N2 washout in small animals and newborn infants. 1985;19:1165-9.
50. Gerhardt T, Reifenberg L, Hehre D, Feller R, Bancalari E. Functional residual capacity in normal neonates and children up to 5 years of age determined by a N2 washout method. Pediatr Res 1986;20:668-71.
51. Sivan Y, Hammer J, Newth CJ. Measurement of high lung volumes by nitrogen washout method. J Appl Physiol 1994;77:1562-4.
52. Sivan Y, Deakers TW, Newth CJ. Functional residual capacity in ventilated infants and children. Pediatr Res 1990;28:451-4.
53. Yuksel B, Greenough A, Chan V, Russell RR. Comparison of helium dilution and nitrogen washout measurements of functional residual capacity in premature infants. Pediatr Pulmonol 1993;16:197-200.
54. Wilke T, Schmalisch G, Wauer RR, Werner C. Accuracy and reproducibility of the N2 washout procedure for the determination of the functional residual capacity in ventilated neonates. PadiatrGrenzgeb 1994;33:373-80.
55. Wilke T, Schmalisch G, Werner C, Wauer RR. [Determination of functional residual capacity by N2 washout procedure.] Bestimmung der funktionellen Residualkapazit"t bei beatmeten Neugeborenen mittels Stickstoff-Auswaschverfahren. 1992;47:139-42.
56. Krause MF, Jakel C, Haberstroh J, Schulte-Monting J, Hoehn T. Functional residual capacity determines the effect of inhaled nitric oxide on intrapulmonary shunt and gas exchange in a piglet model of lung injury. PediatrCrit Care Med 2001;2:82-7.
57. Dinger J, Topfer A, Schaller P, Schwarze R. Functional residual capacity and compliance of the respiratory system after surfactant treatment in premature infants with severe respiratory distress syndrome. Eur J Pediatr 2002;161:485-90.
58. Kavvadia V, Greenough A, Itakura Y, Dimitriou G. Neonatal lung function in very immature infants with and without RDS. 1999;27:382-7.
59. Lichtwarck-Aschoff M, Hedlund AJ, Nordgren KA, et al. Variables used to set PEEP in the lung lavage model are poorly related. BrJ Anaesth 1999;83:890-7.

60. Dinger J, Topfer A, Schaller P, Schwarze R. Effect of positive end expiratory pressure on functional residual capacity and compliance in surfactant-treated preterm infants. J Perinat Med 2001;29:137-43.
61. Dimitriou G, Greenough A, Laubscher B. Appropriate positive end expiratory pressure level in surfactant-treated preterm infants. Eur J Pediatr 1999;158:888-91.
62. Dobbinson TL, Nisbet HI, Pelton DA, Levison H. Functional residual capacity (FRC) and compliance in anaesthetized paralysed children. II. Clinical results. Can Anaesth Soc J 1973;20:322-33.
63. von Ungern-Sternberg BS, Frei FJ, Hammer J, Schibler A, Doerig R, Erb TO. Impact of depth of propofol anaesthesia on functional residual capacity and ventilation distribution in healthy preschool children. BrJ Anaesth 2007;98:503-8.
64. von Ungern-Sternberg BS, Hammer J, Schibler A, Frei FJ, Erb TO. Decrease of functional residual capacity and ventilation homogeneity after neuromuscular blockade in anesthetized young infants and preschool children. Anesthesiology 2006;105:670-5.
65. Greenough A. Clinical application of lung function testing in ventilated infants. Monaldi Arch Chest Dis 1994;49:61-5.
66. Heldt GP, Peters RM. A simplified method to determine functional residual capacity during mechanical ventilation. Chest 1978;74:492-6.
67. Hentschel R, Suska A, Volbracht A, Harms E, Haberland H, Jorch G. Physical effects of heliox versus oxygen on measurements of functional residual capacity by the nitrogen washout technique in small lung volumes: a model study. 2001;31:255-60.
68. Hentschel R, Suska A, Volbracht A, Brune T, Jorch G. Modification of the open circuit N2 washout technique for measurement of functional residual capacity in premature infants. Pediatr Pulmonol 1997;23:434-41.
69. Wauer J, Leier TU, Henschen M, Wauer RR, Schmalisch G. In vitro validation of an ultrasonic flowmeter in order to measure the functional residual capacity in newborns. Physiol Meas 2003;24:355-65.
70. Mahmoud RA, Fischer HS, Proquitte H, Shalaby HM, Schmalisch G. Relationship between endotracheal tube leakage and under-reading of tidal volume in neonatal ventilators. Acta Paediatr 2009;98:1116-22.
71. Mahmoud RA, Proquitte H, Fawzy N, Buhrer C, Schmalisch G. Tracheal tube airleak in clinical practice and impact on tidal volume measurement in ventilated neonates. Pediatr Crit Care Med 2011;12:197-202.
72. Emmen HH, Hoogendijk EM, Klopping-Ketelaars WA, et al. Human safety and pharmacokinetics of the CFC alternative propellants HFC 134a (1,1,1,2-tetrafluoroethane) and HFC 227 (1,1,1,2,3,3, 3-heptafluoropropane) following whole-body exposure. RegulToxicolPharmacol 2000;32:22-35.
73. Koster U, Mayer D, Deger HM, DeKant W. Biotransformation of the aerosol propellant 1,1,1,2,3,3,3-heptafluoropropane (HFA-227): lack of protein binding of the metabolite hexafluoroacetone. Drug Metab Dispos 1996;24:906-10.
74. Dickinson PA, Howells SW, Kellaway IW. Novel nanoparticles for pulmonary drug administration. J Drug Target 2001;9:295-302.
75. Graepel P, Alexander DJ. CFC replacements: safety testing, approval for use in metered dose inhalers. J Aerosol Med 1991;4:193-200.
76. Smyth HD. Propellant-driven metered-dose inhalers for pulmonary drug delivery. Expert Opin Drug Deliv 2005;2:53-74.
77. Auwarter V, Proquitte H, Schmalisch G, Wauer R, Pragst F. Determination of 1,1,1,2,3,3,3-Heptafluoropropane (HFP) in Blood by Headspace Gas Chromatography-Mass Spectrometry. J Anal Toxicol 2005;29:574-6.

78. Proquitte H, Kusztrich A, Auwarter V, Pragst F, Wauer RR, Schmalisch G. Functional residual capacity measurement by heptafluoropropane in ventilated newborn lungs: In vitro and in vivo validation. Crit Care Med 2006;6:1789-95.
79. Daniels S, Paton WD, Smith EB. The effects of some hydrophobic gases on the pulmonary surfactant system. Br J Pharmacol 1979;65:229-35.
80. Lee BR, Lee JD, Lee HJ, et al. Surfactant effects on SF6 hydrate formation. J Colloid Interface Sci 2009;331:55-9.
81. Thome U, Topfer A, Schaller P, Pohlandt F. Comparison of lung volume measurements by antero-posterior chest X-ray and the SF6 washout technique in mechanically ventilated infants. Pediatr Pulmonol 1998;26:265-72.
82. Blondino FE, Byron PR. Surfactant dissolution and water solubilization in chlorine-free liquified gas propellants. Drug Dev Ind Pharm 1998;24:935-45.
83. Martin AR, Kwok DY, Finlay WH. Investigating the evaporation of metered-dose inhaler formulations in humid air: single droplet experiments. J Aerosol Med 2005;18:218-24.
84. Ridder KB, Davies-Cutting CJ, Kellaway IW. Surfactant solubility and aggregate orientation in hydrofluoroalkanes. Int J Pharm 2005;295:57-65.
85. Vervaet C, Byron PR. Drug-surfactant-propellant interactions in HFA-formulations. Int J Pharm 1999;186:13-30.
86. Butz N, Porte C, Courrier H, Krafft MP, Vandamme TF. Reverse water-in-fluorocarbon emulsions for use in pressurized metered-dose inhalers containing hydrofluoroalkane propellants. Int J Pharm 2002;238:257-69.
87. Dickinson PA, Seville PC, McHale H, Perkins NC, Taylor G. An investigation of the solubility of various compounds in the hydrofluoroalkane propellants and possible model liquid propellants. J Aerosol Med 2000;13:179-86.
88. Hlastala MP, Meyer M, Riepl G, Scheid P. Solubility of helium, argon, and sulfur hexafluoride in human blood measured by mass spectrometry. Undersea Biomed Res 1980;7:297-304.
89. Ohta Y, Ar A, Farhi LE. Solubility and partition coefficients for gases in rabbit brain and blood. J Appl Physiol 1979;46:1169-70.
90. Smith RA, Porter EG, Miller KW. The solubility of anesthetic gases in lipid bilayers. Biochim Biophys Acta 1981;645:327-38.
91. Young IH, Wagner PD. Solubility of inert gases in homogenates of canine lung tissue. J Appl Physiol 1979;46:1207-10.
92. Pilling KJ, Jones HW. Inhalation of degraded sulphur hexafluoride resulting in pulmonary oedema. J Soc Occup Med 1988;38:82-4.
93. Juzoji H, Iwasaki T, Usui M, Hasemi M, Yamakawa N. Histological study of intraocular changes in rabbits after intravitreal gas injection. Jpn J Ophthalmol 1997;41:278-83.
94. Landry H, Aminian A, Hoffart L, et al. Corneal endothelial toxicity of air and SF6. Invest Ophthalmol Vis Sci 2011;52:2279-86.
95. Schulze F, Schmidtsdorf H. [Damage to the corneal endothelium following exposure to sulfur hexafluoride gas]. Klin Monbl Augenheilkd 1989;194:447-53.
96. Sztarbala T, Gos R, Goralczyk M, Kedziora J, Blaszczyk J, Sibinska E. Changes in the structure and properties of corpus vitreous and the preservation of enzymatic composition of rabbit antioxidative system under the influence of sulphur hexafluoride gas. Med Sci Monit 2000;6:240-3.
97. Vinegar A, Jepson GW. Cardiac sensitization thresholds of halon replacement chemicals predicted in humans by physiologically-based pharmacokinetic modeling. Risk Anal 1996;16:571-9.

98. Dervos CT, Vassiliou P. Sulfur hexafluoride (SF6): global environmental effects and toxic byproduct formation. J Air Waste Manag Assoc 2000;50:137-41.
99. Gentil E, Christensen TH, Aoustin E. Greenhouse gas accounting and waste management. Waste Manag Res 2009;27:696-706.
100. Harnisch J, Hohne N. Comparison of emissions estimates derived from atmospheric measurements with national estimates of HFCs, PFCs and SF6. Environ Sci Pollut Res Int 2002;9:315-20.
101. Pelc A. Generation of negative ions from SF(6) gas by means of hot surface ionization. Rapid Commun Mass Spectrom 2012;26:577-82.
102. Schibler A, Schneider M, Frey U, Kraemer R. Moment ratio analysis of multiple breath nitrogen washout in infants with lung disease. Eur Respir J 2000;15:1094-101.
103. Schibler A, Henning R. Measurement of functional residual capacity in rabbits and children using an ultrasonic flow meter. Pediatr Res 2001;49:581-8.
104. Frey U, Stocks J, Coates A, Sly P, Bates J. Specifications for equipment used for infant pulmonary function testing. ERS/ATS Task Force on Standards for Infant Respiratory Function Testing. European Respiratory Society/ American Thoracic Society. Eur Respir J 2000;16:731-40.
105. Frey U, Stocks J, Sly P, Bates J. Specification for signal processing and data handling used for infant pulmonary function testing. ERS/ATS Task Force on Standards for Infant Respiratory Function Testing. European Respiratory Society/American Thoracic Society. Eur Respir J 2000;16:1016-22.
106. Miller J, Law AB, Parker RA, Sundell HW, Lindstrom DP, Cotton RB. Validation of a nitrogen washout system to measure functional residual capacity in premature infants with hyaline membrane disease. Pediatr Pulmonol 1995;20:403-9.
107. Pillow JJ, Ljungberg H, Hulskamp G, Stocks J. Functional residual capacity measurements in healthy infants: ultrasonic flow meter versus a mass spectrometer. Eur Respir J 2004;23:763-8.
108. Schibler A, Frey U. Role of lung function testing in the management of mechanically ventilated infants. Arch Dis Child Fetal Neonatal Ed 2002;87:F7-F10.
109. Schmalisch G, Proquitte H, Roehr CC, Wauer RR. The effect of changing ventilator settings on indices of ventilation inhomogeneity in small ventilated lungs. BMC Pulm Med 2006;6:20.
110. Schibler A, Henning R. Positive end-expiratory pressure and ventilation inhomogeneity in mechanically ventilated children. Pediatr Crit Care Med 2002;3:124-8.
111. Shao H, Sandberg K, Sjoqvist BA, Hjalmarson O. Moment analysis of multibreath nitrogen washout in healthy preterm infants. Pediatr Pulmonol 1998;25:52-8.
112. Schibler A, Hall GL, Businger F, et al. Measurement of lung volume and ventilation distribution with an ultrasonic flow meter in healthy infants. Eur Respir J 2002;20:912-8.
113. Aurora P, Gustafsson P, Bush A, et al. Multiple breath inert gas washout as a measure of ventilation distribution in children with cystic fibrosis. Thorax 2004;59:1068-73.
114. Kraemer R, Blum A, Schibler A, Ammann RA, Gallati S. Ventilation inhomogeneities in relation to standard lung function in patients with cystic fibrosis. Am J Respir Crit Care Med 2005;171:371-8.
115. Aurora P, Bush A, Gustafsson P, et al. Multiple-breath washout as a marker of lung disease in preschool children with cystic fibrosis. Am J Respir Crit Care Med 2005;171:249-56.
116. Hjalmarson O, Sandberg KL. Lung function at term reflects severity of bronchopulmonary dysplasia. J Pediatr 2005;146:86-90.

117. Gustafsson PM, Aurora P, Lindblad A. Evaluation of ventilation maldistribution as an early indicator of lung disease in children with cystic fibrosis. Eur Respir J 2003;22:972-9.
118. Kiorpes AL, Clayton MK. Moment analysis of multibreath nitrogen washout in healthy female goats and calves. Am J Vet Res 1988;49:543-7.
119. Bates JH, Schmalisch G, Filbrun D, Stocks J. Tidal breath analysis for infant pulmonary function testing. ERS/ATS Task Force on Standards for Infant Respiratory Function Testing. European Respiratory Society/American Thoracic Society. Eur Respir J 2000;16:1180-92.
120. Beydon N, Davis SD, Lombardi E, et al. An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children. Am J Respir Crit Care Med 2007;175:1304-45.
121. Shao H, Sandberg K, Hjalmarson O. Impaired gas mixing and low lung volume in preterm infants with mild chronic lung disease. Pediatr Res 1998;43:536-41.
122. von Ungern-Sternberg BS, Regli A, Frei FJ, et al. A deeper level of ketamine anesthesia does not affect functional residual capacity and ventilation distribution in healthy preschool children. Paediatric anaesthesia 2007;17:1150-5.
123. Pham TM, Yuill M, Dakin C, Schibler A. Regional ventilation distribution in the first 6 months of life. Eur Respir J 2011;37:919-24.
124. Proquitte H, Krause S, Rudiger M, Wauer RR, Schmalisch G. Current limitations of volumetric capnography in surfactant-depleted small lungs. Pediatr Crit Care Med 2004;5:75-80.
125. Elgeti T, Proquitte H, Rogalla NE, et al. Dynamic computed tomography of the neonatal lung: volume calculations and validation in an animal model. Invest Radiol 2005;40:761-5.
126. Elgeti T, Proquitte H, Rogalla NE, et al. Evaluation of a reduced dose protocol for respiratory gated lung computed tomography in an animal model. Invest Radiol 2007;42:230-4.
127. Proquitte H, Elgeti T, Roehr CC, Rogalla P, Wauer RR, Schmalisch G. Comparison of lung volume measurements by multiple breath heptafluoropropane washout and computed tomography in small ventilated lungs. Med Sci Monit 2009;15:20-4.
128. Chiumello D, Cressoni M, Chierichetti M, et al. Nitrogen washout/washin, helium dilution and computed tomography in the assessment of end expiratory lung volume. Crit Care 2008;12:R150.
129. Kauczor HU, Heussel CP, Fischer B, Klamm R, Mildenberger P, Thelen M. Assessment of lung volumes using helical CT at inspiration and expiration: comparison with pulmonary function tests. AJR Am J Roentgenol 1998;171:1091-5.
130. Kauczor HU, Hast J, Heussel CP, Schlegel J, Mildenberger P, Thelen M. CT attenuation of paired HRCT scans obtained at full inspiratory/expiratory position: comparison with pulmonary function tests. Eur Radiol 2002;12:2757-63.
131. Gattinoni L, Tognoni G, Pesenti A, et al. Effect of prone positioning on the survival of patients with acute respiratory failure. N Engl J Med 2001;345:568-73.
132. Gattinoni L, Pesenti A. The concept of "baby lung". Intensive Care Med 2005;31:776-84.
133. Gattinoni L, Pelosi P, Vitale G, Pesenti A, D'Andrea L, Mascheroni D. Body position changes redistribute lung computed-tomographic density in patients with acute respiratory failure. Anesthesiology 1991;74:15-23.
134. Guerin C. Ventilation in the prone position in patients with acute lung injury/acute respiratory distress syndrome. Curr Opin Crit Care 2006;12:50-4.

135. Curley MA, Thompson JE, Arnold JH. The effects of early and repeated prone positioning in pediatric patients with acute lung injury. Chest 2000;118:156-63.
136. Curley MA, Hibberd PL, Fineman LD, et al. Effect of prone positioning on clinical outcomes in children with acute lung injury: a randomized controlled trial. Jama 2005;294:229-37.
137. Kumar P, Leonidas JC, Ashtari M, Napolitano B, Steele AM. Comparison of lung area by chest radiograph, with estimation of lung volume by helium dilution during prone and supine positioning in mechanically ventilated preterm infants: a pilot study. Pediatr Pulmonol 2005;40:219-22.
138. Numa AH, Hammer J, Newth CJ. Effect of prone and supine positions on functional residual capacity, oxygenation, and respiratory mechanics in ventilated infants and children. Am J Respir Crit Care Med 1997;156:1185-9.
139. Fox RE, Viscardi RM, Taciak VL, Niknafs H, Cinoman MI. Effect of position on pulmonary mechanics in healthy preterm newborn infants. J Perinatol 1993;13:205-11.
140. Larsson A, Gilbert JT, Bunegin L, Gelineau J, Smith RB. Pulmonary effects of body position, PEEP, and surfactant depletion in dogs. Acta Anaesthesiol Scand 1992;36:38-45.
141. Proquitte H, Freiberger O, Yilmaz S, et al. The effect of surgery on lung volume and conventional monitoring parameters in ventilated newborn infants. Eur Respir J 2010;35:1072-8.
142. Pillow JJ, Frerichs I, Stocks J. Lung function tests in neonates and infants with chronic lung disease: global and regional ventilation inhomogeneity. Pediatr Pulmonol 2006;41:105-21.
143. Gucciardo L, Deprest J, Done E, et al. Prediction of outcome in isolated congenital diaphragmatic hernia and its consequences for fetal therapy. Best practice & research 2008;22:123-38.
144. Peralta CF, Jani J, Cos T, Nicolaides KH, Deprest J. Left and right lung volumes in fetuses with diaphragmatic hernia. Ultrasound Obstet Gynecol 2006;27:551-4.
145. Peralta CF, Jani JC, Van Schoubroeck D, Nicolaides KH, Deprest JA. Fetal lung volume after endoscopic tracheal occlusion in the prediction of postnatal outcome. American journal of obstetrics and gynecology 2008;198:60 e1-5.
146. Matthews IL, Kaldestad RH, Bjornstad PG, Thaulow E, Gronn M. Preoperative lung function in newborn infants with univentricular hearts compared with healthy controls. Acta Paediatr 2007;96:44-8.
147. Matthews IL, Kaldestad RH, Bjornstad PG, Thaulow E, Gronn M. Differing lung function development in infants with univentricular hearts compared with healthy infants. Acta Paediatr 2008;97:1645-52.
148. Nakayama DK, Mutich R, Motoyama EK. Pulmonary dysfunction after primary closure of an abdominal wall defect and its improvement with bronchodilators. Pediatr Pulmonol 1992;12:174-80.
149. Nakayama DK, Mutich R, Motoyama EK. Pulmonary dysfunction in surgical conditions of the newborn infant. Crit Care Med 1991;19:926-33.
150. DiCarlo JV, Raphaely RC, Steven JM, Norwood WI, Costarino AT. Pulmonary mechanics in infants after cardiac surgery. Crit Care Med 1992;20:22-7.
151. Dimitriou G, Greenough A, Giffin F, Davenport M, Nicolaides KH. Temporary impairment of lung function in infants with anterior abdominal wall defects who have undergone surgery. J Pediatr Surg 1996;31:670-2.

152. Laubscher B, Greenough A, Dimitriou G, Davenport M, Nicolaides KH. Serial lung volume measurements during the perinatal period in infants with abdominal wall defects. J Pediatr Surg 1998;33:497-9.
153. Dinger J, Peter-Kern M, Goebel P, Roesner D, Schwarze R. Effect of PEEP and suction via chest drain on functional residual capacity and lung compliance after surgical repair of congenital diaphragmatic hernia: preliminary observations in 5 patients. J Pediatr Surg 2000;35:1482-8.
154. Boloker J, Bateman DA, Wung JT, Stolar CJ. Congenital diaphragmatic hernia in 120 infants treated consecutively with permissive hypercapnea/spontaneous respiration/elective repair. J Pediatr Surg 2002;37:357-66.
155. Roehr CC, Proquitté H, Jung A, et al. Impaired somatic growth and delayed lung development in infants with congenital diaphragmatic hernia - evidence from a 10-year, single center prospective follow-up study. J Pediatr Surg 2009;7:1309-14.
156. Hulskamp G, Lum S, Stocks J, et al. Association of prematurity, lung disease and body size with lung volume and ventilation inhomogeneity in unsedated neonates: a multicentre study. Thorax 2009;64:240-5.
157. Seidenberg J, Homberger J, von der Hardt H. Effect of endotracheal tube leakage on functional residual capacity determination by nitrogen washout method in a small-sized lung model. Pediatr Pulmonol 1994;17:106-12.
158. Fox WW, Schwartz JG, Shaffer TH. Effects of endotracheal tube leaks on functional residual capacity determination in intubated neonates. Pediatr Res 1979;13:60-4.
159. Bernstein G, Knodel E, Heldt GP. Airway leak size in neonates and autocycling of three flow-triggered ventilators. Crit Care Med 1995;23:1739-44.
160. Edberg KE, Sandberg K, Silberberg A, Ekstrom-Jodal B, Hjalmarson O. Lung volume, gas mixing, and mechanics of breathing in mechanically ventilated very low birth weight infants with idiopathic respiratory distress syndrome. Pediatr Res 1991;30:496-500.
161. Schwartz JG, Fox WW, Shaffer TH. A method for measuring functional residual capacity in neonates with endotracheal tubes. IEEE Trans Biomed Eng 1978;25:304-7.
162. von Ungern-Sternberg BS, Hammer J, Frei FJ, Jordi Ritz EM, Schibler A, Erb TO. Prone equals prone? Impact of positioning techniques on respiratory function in anesthetized and paralyzed healthy children. Intensive Care Med 2007;33:1771-7.

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7. Declaration instead of oath

Eidesstattliche Erklärung

§ 4 Abs. 3 (k) der Habilitationsordnung der Medinizinischen Fakultät Charité

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wird bzw. wurde,
- die vorgelegte Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Ergebnisse selbst gewonnen sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlern / Wissenschaftlerinnen und mit technischen Hilfskräften sowie die verwendete Literatur vollständig in der Habilitationsschrift angegeben wurden und
- mir die geltende Habilitationsordnung bekannt ist.

Dr. Hans Proquitté

Berlin, den 02.03.2012