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Laboratory Investigation

LABORATORY INVESTIGATION

Validating the inspired sinewave technique to measure the volume of the 'baby lung' in a porcine lung-injury model

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Abstract

Background: Bedside lung volume measurement could personalise ventilation and reduce driving pressure in patients with acute respiratory distress syndrome (ARDS). We investigated a modified gas-dilution method, the inspired sinewave technique (IST), to measure the effective lung volume (ELV) in pigs with uninjured lungs and in an ARDS model.

Methods: Anaesthetised mechanically ventilated pigs were studied before and after surfactant depletion by saline lavage. Changes in PEEP were used to change ELV. Paired measurements of absolute ELV were taken with IST (ELV_{IST}) and compared with gold-standard measures (sulphur hexafluoride wash in/washout [ELV_{SF6}] and computed tomography (CT) [ELV_{CT}]). Measured volumes were used to calculate changes in ELV (ΔELV) between PEEP levels for each method (ΔELV_{IST}, ΔELV_{SF6}, and ΔELV_{CT}).

Results: The coefficient of variation was <5% for repeated ELV_{IST} measurements (n=13 pigs). There was a strong linear relationship between ELV_{IST} and ELV_{SF6} in uninjured lungs (r^2=0.97), and with both ELV_{SF6} and ELV_{CT} in the ARDS model (r^2=0.87 and 0.92, respectively). ELV_{IST} had a mean bias of −12 to 13% (95% limits=±17 − 25%) compared with ELV_{SF6} and ELV_{CT}. ΔELV_{IST} was concordant with ΔELV_{SF6} and ΔELV_{CT} in 98–100% of measurements, and had a mean bias of −73 to −77 ml (95% limits=±128 − 186 ml) compared with ΔELV_{SF6} and −1 ml (95% limits ±333 ml) compared with ΔELV_{CT}.

Conclusions: IST provides a repeatable measure of absolute ELV and shows minimal bias when tracking PEEP-induced changes in lung volume compared with CT in a saline-lavage model of ARDS.

Keywords: ARDS; computed tomography; inspired sinewave technique; lung volume measurement; method comparison

Editor's key points

- The development of lung volume measurements at the bedside may enable personalised ventilatory strategies that optimise respiratory function in acute lung injury.
- The authors used a modified gas-dilution method—the inspired sinewave technique—to measure effective lung volume measured by CT in mechanically ventilated pigs with uninjured lungs and acute respiratory distress syndrome (ARDS) induced by saline lavage.
- The inspired sinewave technique provided a repeatable measure of absolute effective lung volume.
- This individualised measurement approach may help reduce ventilator-induced injury during ARDS and acute lung injury.

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The acute respiratory distress syndrome (ARDS) affects 10% of critical care patients and has an associated mortality of 40%.[1] Patients with ARDS have a smaller aerated lung volume ('baby lung') and one of the few interventions shown to reduce mortality in ARDS is ventilating patients with 6–8 ml kg^{-1} tidal volume (V_T).[2–4] More recently, driving pressure (ΔP) has been shown to be the ventilator parameter that best stratifies risk of mortality in ARDS.[5] ΔP is dependent on V_T and respiratory system compliance (C_{RS}) ($\Delta P = V_T/C_{RS}$). C_{RS} is dependent on the size of the baby lung, and therefore, so too is ΔP.[6] It is thought that reduced mortality from low V_T ventilation may be secondary to reducing the effect that ΔP has on the ARDS baby lung.[3]

Whilst it might be possible to titrate V_T to ΔP,[7] the underlying volume of the baby lung remains the fundamental parameter on which the imposed V_T exerts its effect to produce ΔP. Therefore, there would be potential benefit in measuring the volume of lung of the baby lung at the bedside of a mechanically ventilated ARDS patient. This would allow personalised titration of V_T to the underlying lung volume, with limitation of lung strain caused by ΔP. Lung volume can be measured in ventilated patients by imaging (CT), wash-in/washout techniques, or gas-dilution techniques. CT imaging puts patients at risk by necessitating a transfer to the scanner and exposure to ionising radiation. The current bedside gas-based techniques have not proved reliable in assisted ventilation, or they require equipment that is too bulky or too time-consuming for bedside clinical use.[8–13]

The inspired sinewave technique (IST) has the potential to provide bedside measurement of the lung volume, which takes part in gas exchange (effective lung volume [ELV]). IST uses a low concentration of tracer gas (<5% nitrous oxide [N_2O]) injected into the inspired airway gases in a sinusoidally oscillating concentration.[14–16] Sinewave amplitude and phase of the concentration of the tracer gas measured in the expiratory airway gases are used to recover values of ELV (ELV_{IST}) and pulmonary blood flow. IST uses commercially available components commonly used in anaesthetic/intensive care practice, and has proven efficacy in spontaneously ventilating healthy volunteers.[17,18] To date, IST's ability to measure ELV in mechanically ventilated lungs has not been tested.

The aim of the current study was to conduct a method comparison, measuring the relationship and limits of agreement between ELV measured by IST compared with SF_6 wash in/washout and CT. To do this, we compared the absolute ELV and changes in ELV (ΔELV) at and between different PEEPs in mechanically ventilated pigs both before and after induction of ARDS by saline lavage.[10,19,20]

Table 1 Baseline characteristics for n=13 animals pre- and post-lung injury. Mean (standard deviation) are shown for parametric data and median (95% confidence interval) for non-parametric data. P-values in the final column represent results of either paired Student's t-test (of parametric data) or Wilcoxon signed rank test (of non-parametric data). CO, cardiac output; DBP, diastolic BP; FIO_2, fraction of inspired O_2; Hb, haemoglobin; $PaCO_2$, arterial CO_2 partial pressure; PADP, pulmonary artery diastolic BP; PaO_2, arterial O_2 partial pressure; PASP, pulmonary artery systolic pressure; PFR, PaO_2:FIO_2 ratio; SaO_2, arterial oxygen saturation; SBP, systolic BP.

Parameter	Pre-injury	Post-injury	P-value
Weight	29 (2)	—	
HR (bpm)	86 (70–100)	85 (80–95)	0.42
SBP (mm Hg)	100 (8)	98 (14)	0.57
DBP (mm Hg)	67 (12)	54 (8)	0.0006
CO (L min^{-1})	3.4 (0.8)	3.2 (0.4)	0.26
PASP (mm Hg)	28 (25–33)	32 (29–36)	0.03
PADP (mm Hg)	16 (4)	19 (5)	0.004
Hb (g dl^{-1})	83 (5)	84 (6)	0.93
FIO_2	0.4 (0.3–0.4)	0.8 (0.7–0.9)	0.0002
SaO_2 (%)	100 (99–100)	98 (91–99)	0.001
pH	7.38 (0.07)	7.25 (0.08)	0.0004
PaO_2 (kPa)	19.2 (15.9–21.5)	12.8 (11.3–26.0)	0.39
$PaCO_2$ (kPa)	6.7 (1.1)	8.4 (2.0)	0.003
PFR	377 (304–513)	128 (101–248)	0.0002

Methods

Ethical approval

This study of 13 domestic pigs (mean weight [standard deviation {SD}]=29 [2] kg) at the Hedenstierna Laboratory, Uppsala

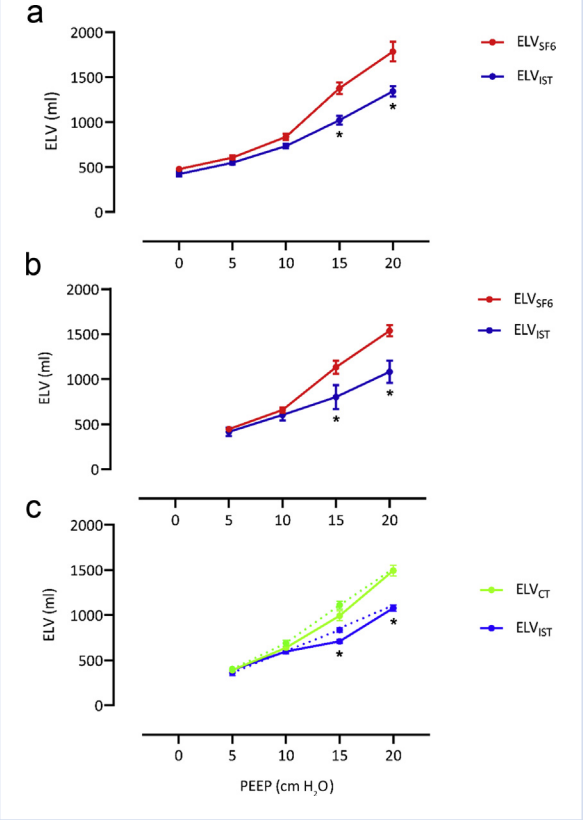


Fig 1. Effective lung volume (ELV) measurement (standard error) using ELV_{IST}, ELV_{SF6}, and ELV_{CT} at each PEEP level. (a) Paired ELV_{IST} and ELV_{SF6} measurements in the uninjured lung. (b) Paired ELV_{IST} and ELV_{SF6} measurements in the acute respiratory distress syndrome (ARDS) model. (c) Paired ELV_{IST} and ELV_{CT} measurements in the ARDS model. PEEP=0 cm H_2O was not studied post-injury; PEEP levels of 15 and 20 cm H_2O were studied in six of the total n=13 animals. Solid lines represent volumes measured during incremental PEEP titration; dashed lines represent volumes measured during decremental PEEP titration. *ELV_{IST} is significantly different to ELV_{SF6} or ELV_{CT} (P>0.05). Table 2 provides full details of volumes measured and probabilities of difference.

University, Uppsala, Sweden was approved by the regional animal welfare and ethics committee (Ref: C98/16). Reporting in this paper adheres to the Animal Research: Reporting of In Vivo Experiments guidelines.[21]

Animal preparation

Table 1 shows the summary baseline characteristics of the animals pre- and post-lung injury (individual values are given in Supplementary Table 1). The animals were prepared and anaesthetised using i.m. sedation and subsequent i.v. anaesthesia as described elsewhere.[22] During preparation, mechanical ventilation was delivered in volume-controlled ventilation (VCV) mode at 20–25 bpm (to maintain end-tidal CO_2 [$EtCO_2$] between 4.5 and 6 kPa), with a V_T of 10 ml kg^{-1}, PEEP of 5 cm H_2O, and an inspiratory:expiratory ratio (I:E) of 1:2 (Servo-I®; Maquet, Rastatt, Germany). Airway leaks were excluded by analysis of spirometry data. Depth of anaesthesia was confirmed by lack of spontaneous movements, absence of reaction to painful stimulation between the front hooves, and absence of cardiovascular signs of sympathetic stimulation (increases in HR or arterial BP). Subsequent muscle relaxation was achieved with a bolus of rocuronium 0.2 mg kg^{-1} followed by 0.1 mg kg^{-1} boluses when spontaneous ventilatory efforts were detected from the airway gas and pressure traces. Maintenance i.v. crystalloid fluids were administered (Ringerfundin®; B. Braun Melsungen AG, Melsungen, Germany) at a rate of 10 ml kg^{-1} h^{-1} during the preparation phase and 7 ml kg^{-1} h^{-1} for the rest of the protocol. Once anaesthetised, the right internal jugular vein was cannulated with a pulmonary artery catheter used for continuous pulmonary artery pressure, intermittent thermodilution cardiac output, and core temperature monitoring.

Data collection and processing

Cardiorespiratory variables, including peripheral oxyhaemoglobin saturation (SpO_2), ECG, invasive arterial BP (AS/3 Multi-Parameter Patient Monitor, Datex-Ohmeda, Madison, WI, USA), airway gas composition, flow, and pressure (Capnomac Ultima™; Datex-Ohmeda) were continuously monitored and acquired as analogue signals throughout the protocol. Analogue data were converted using PowerLab (ADInstruments, Dunedin, New Zealand) and displayed/recorded with LabChart version 8.1.5 (ADInstruments). Physiological data were processed using R version 3.5.1 (R Core Team (2017), Vienna, Austria).[23]

Inspired sinewave technique

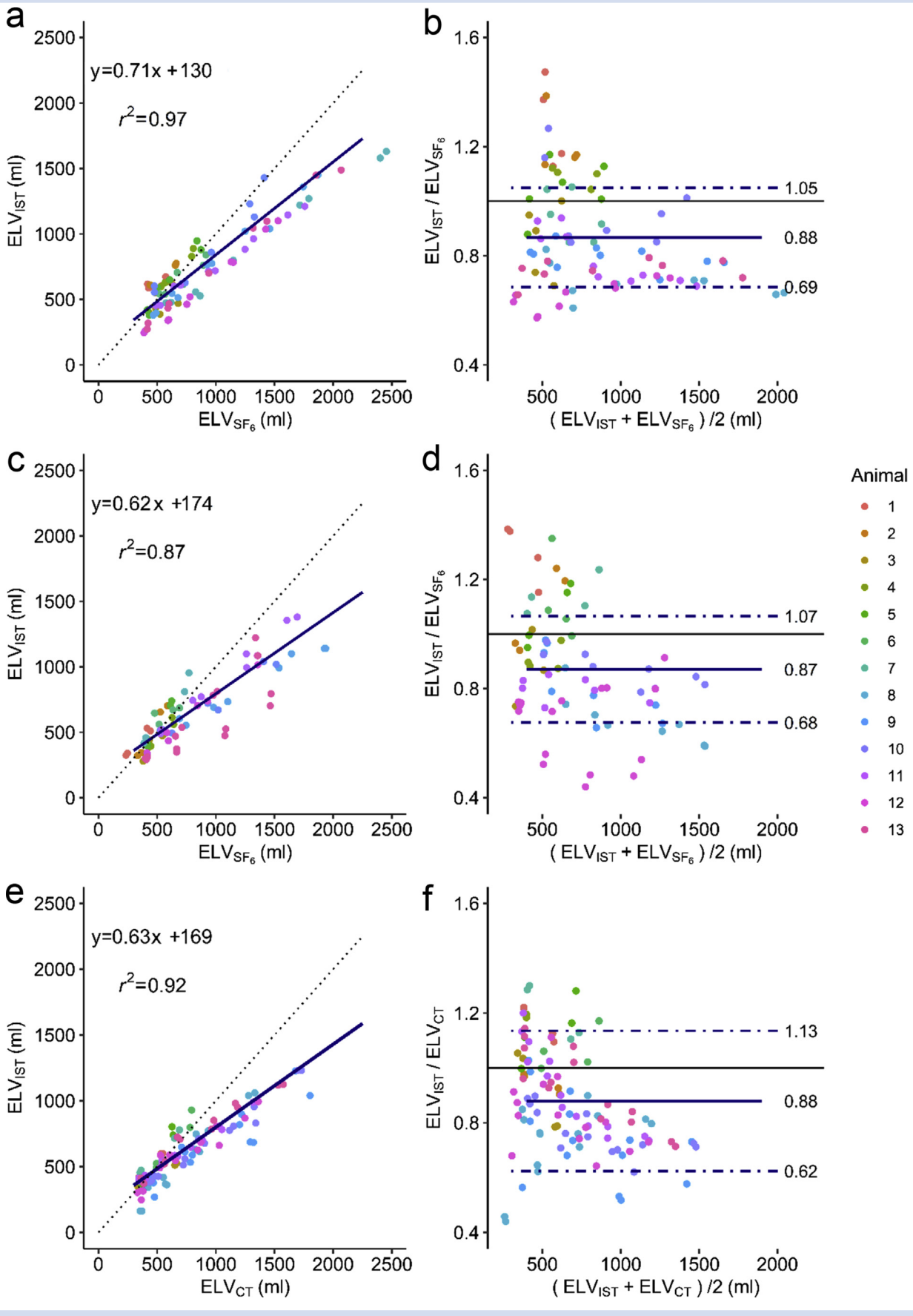
A mainstream infrared N_2O and CO_2 sensor (IRMA, Danderyd, Thor Laboratories, Budapest Sweden) and an ultrasonic flowmeter (VenThor 22/2A; Thor Laboratories, Budapest, Hungary) were placed in the breathing circuit between a patient-end heat-moisture exchange filter (Intersurgical, Wokingham, UK) and the ventilator tubing. At the start of each inspiration, a set volume of N_2O was injected into the inspired gas by a mass flow controller (Alicat Scientific, Tucson, AZ, USA). The volume of N_2O injected was proportional to the inspiratory flow and oscillated sinusoidally over consecutive breaths around a set mean concentration of 5%, with an amplitude of 4% and a period of 180 s for a duration of 270 s. The test duration of 270 s was the maximum duration that would allow repetition of each test within the permitted time frame of the protocol. By measuring the expired N_2O concentration in each consecutive breath, a set of simultaneous equations can be solved to provide estimates of ELV. Further technical details of IST are described elsewhere.[15–17,24]

SF_6 wash in/washout

The use of SF_6 as a tracer gas for wash-in/washout measurement of lung volume is detailed elsewhere.[10] The apparatus was attached between the tracheal tube and the D-lite™ spirometer attachment (GE Healthcare, Chicago, IL, USA).

Table 2 Descriptive statistics for comparisons of absolute effective lung volume (ELV) and changes in ELV (ΔELV) made in both the uninjured lung and the acute respiratory distress syndrome (ARDS) model at each PEEP level. Mean (standard deviation) volumes are shown in millilitres. n, number of observations (incomplete data sets have been excluded from the analysis; these account for 4% of total possible paired measurements). ELV_{CT}, CT measured absolute volume; ELV_{IST}, inspired sinewave technique (IST) measured absolute volume; ELV_{SF6}, SF_6 measured absolute volume; P, probability from either two-way analysis of variance or Kruskal–Wallis that mean measurements at each PEEP level are from different samples; ΔELV_{CT}, CT measured change in lung volume; ΔELV_{IST}, IST measured change in lung volume; ΔELV_{SF6}, SF_6 measured change in lung volume.

PEEP (cm H_2O)	Uninjured				ARDS model							
	n	ELV_{IST}	ELV_{SF6}	*P*-value	*n*	ELV_{IST}	ELV_{SF6}	*P*-value	*n*	ELV_{IST}	ELV_{CT}	*P*-value
0	17	422 (106)	477 (66)	0.35	—	—	—	—	—	—	—	—
5	24	548 (99)	607 (119)	0.31	25	415 (108)	444 (113)	0.84	38	381 (76)	394 (49)	0.86
10	22	734 (132)	837 (169)	0.14	26	601 (149)	657 (157)	0.26	36	598 (124)	654 (99)	0.14
15	11	1022 (159)	1377 (213)	0.002	12	801 (207)	1132 (249)	<0.0001	24	771 (107)	1051 (177)	<0.0001
20	12	1342 (194)	1785 (373)	0.01	12	1081 (197)	1535 (214)	<0.0001	12	1077 (113)	1493 (199)	<0.0001
	n	ΔELV_{IST}	ΔELV_{SF6}	*P*-value	*n*	ΔELV_{IST}	ΔELV_{SF6}	*P*-value	*n*	ΔELV_{IST}	ΔELV_{CT}	*P*-value
0 to >5	17	118 (60)	180 (57)	0.02	—	—	—	—	—	—	—	—
5 to >10	24	190 (87)	240 (71)	0.13	26	191 (127)	216 (91)	0.81	24	209 (105)	245 (58)	0.60
10 to >15	11	313 (90)	423 (111)	0.07	12	252 (73)	399 (91)	0.0008	12	196 (77)	371 (90)	0.0002
15 to >20	12	312 (92)	412 (233)	0.56	12	281 (102)	403 (70)	0.008	12	370 (88)	500 (75)	0.005
20 to >15	—	—	—	—	—	—	—	—	12	−243 (92)	−383 (100)	0.01
15 to >10	—	—	—	—	—	—	—	—	12	−233 (69)	−422 (67)	<0.0001
10 to >5	—	—	—	—	—	—	—	—	12	−242 (51)	−287 (64)	0.37


a
y=0.71x +130
r²=0.97
ELVIST (ml)
ELVSF6 (ml)
b
ELVIST / ELVSF6
(ELVIST + ELVSF6) /2 (ml)
1.05
0.88
0.69
c
y=0.62x +174
r²=0.87
d
1.07
0.87
0.68
Animal
1
2
3
4
5
6
7
8
9
10
11
12
13
e
y=0.63x +169
r²=0.92
ELVCT (ml)
f
ELVIST / ELVCT
(ELVIST + ELVCT) /2 (ml)
1.13
0.88
0.62

CT image acquisition and analysis

A Somatom Definition Flash (Siemens, Munich, Germany) was used to acquire images as a series of transverse sections with a reconstituted voxel size of 0.5×0.5×5 mm. Whole-lung volume scans were conducted during a 20 s end-expiratory pause without ventilator disconnection. Scans were taken using a tube voltage of 80 kV, 364 mA current, and 64×60 mm collimation. Reconstituted whole-lung scans were segmented using 3D Slicer version 4.10.2[25] (https://www.slicer.org/). The mediastinum, diaphragm, inferior vena cava, and hilar vessels were not included in segmentation. Exclusion of intrapulmonary vessels within regions of increased voxel density was not possible, as they were not distinguishable from lung tissue, and these, along with the conducting airways up to the level of the clavicles, were included in the analysis. The aerated volume of each whole-lung scan was calculated from the Hounsfield unit (HU) density of each voxel according to the formula:[26]

$$\text{Aerated volume} = \frac{-\text{mean CT density (HU)}}{1000} \times \text{segmented volume}$$

ARDS model

Lung injury was induced with a technique modified from Lachmann and colleagues.[27] Following preoxygenation, the ventilator was disconnected and the lungs lavaged by instillation of 0.9 % saline solution (at 37°C) via the tracheal tube. After 30 s, the saline was drained out of the lungs and ventilation recommenced. This process was repeated until a PaO_2:FIO_2 ratio of less than 300 mm Hg was achieved.

Study protocol

Throughout all phases of the protocol, the animals were positioned in dorsal recumbency; FIO_2 was titrated to PaO_2 >10 kPa; and the animals were mechanically ventilated with VCV, V_T 10 ml kg^{-1}, and I:E 1:2. The ventilatory frequency was adjusted to 15–20 bpm to maintain $EtCO_2$ <8 kPa. In all cases, measurements were taken after 2 min of ventilation at the studied PEEP level, and the tracheal tube was clamped during airway disconnection (e.g. to change equipment for measuring ELV_{IST} to ELV_{SF6}). Supplementary Figure S1 shows a summary timeline of each experiment.

Comparison between ELV_{IST} and ELV_{SF6} pre- and post-injury

Measurements of ELV were taken with IST (ELV_{IST}) and SF_6 wash in/washout (ELV_{SF6}). Two sets of paired measurements were taken at each PEEP level studied. Measurements were taken first with IST, and then with SF_6. After completion of experiments in the uninjured model, lung injury was induced as described previously, and a further two paired measurements of ELV_{IST} and ELV_{SF6} were repeated at each PEEP level. In all 13 animals, the studied PEEP levels were 0, 5, and 10 cm H_2O. In six of these animals, PEEP levels of 15 and 20 cm H_2O were additionally included. In all comparisons of ELV_{IST} and ELV_{SF6}, PEEP was incrementally increased from low to high. The protocol comparing ELV_{IST} and ELV_{SF6} was the same pre- and post-saline lavage, except that 0 cm H_2O PEEP was not studied post-injury because of the risk that this would not be tolerated.

Comparison between ELV_{IST} and ELV_{CT} after lung injury

Upon completion of the ELV_{IST} *vs* ELV_{SF6} comparisons, the animals were transferred to the CT scanner. Two paired measurements of ELV_{IST} and ELV estimated from CT (ELV_{CT}) were made at each PEEP level. In all 13 animals, the PEEP levels studied were 5 and 10 cm H_2O. In six of these animals, PEEP was titrated from 5 cm H_2O up to 20 cm H_2O, and then back down again. For the reason stated previously, zero PEEP was not studied post-injury. Upon completion of the study protocol, the anaesthetised pigs were euthanised with potassium chloride.

Statistical analyses

Paired measurements of ELV_{IST}, ELV_{SF6}, and ELV_{CT} at each PEEP level were tested for normality using D'Agostino–Pearson's test and QQ plots. Datasets were compared with a repeated measures, two-way analysis of variance (ANOVA) with Sidak multiple comparison test. ΔELV was calculated by subtracting one absolute ELV from its value at the preceding PEEP level. The linear relationship between measurements was tested using linear mixed effects modelling with variation caused by different animals considered a random effect. Conditional r^2 values based on the entire model are reported.[28] The agreement in measurements between techniques was assessed with Bland–Altman analysis corrected for multiple comparisons using a single-factor ANOVA with individual animal as the factor.[29–31] Non-normally distributed data were transformed (by using a ratio of measurements) to conform to the assumption of normality, and 95% limits of agreement were calculated as 1.96 SD from the mean bias. Measured changes in lung volume were compared using four quadrant plots with direction of change concordance and polar plots.[32] For polar plot analysis of trends, adequate limits of agreement are assumed to be within 30° of the horizontal. Analyses were carried out using R version 3.5.1 (R Core Development Team, Vienna, Austria),[23] GraphPad Prism version 8.1.2 (GraphPad Software, La Jolla, CA, USA; https://www.graphpad.com/), and SigmaPlot version 14.0 (Systat Software, San Jose, CA, USA).

Results

A total of n=271 paired ELV measurements in 13 pigs at five different PEEP levels were analysed. Figure 1 shows that the

Fig 2. Scatter and Bland–Altman plots for absolute volume measurements (ELV_{IST}, ELV_{SF6}, and ELV_{CT}). (a, b) Measurements from the uninjured lung. (c–f) Experiments conducted in the acute respiratory distress syndrome model. In the left column, scatterplots show the results of mixed effects linear models, where the solid line represents the reported equation. In the right column, solid lines in the Bland–Altman plots represent the mean bias, and the dashed lines show the upper and lower limits of agreement (±1.96 standard deviation). In all plots, individual points represent a paired set of measurements, and different colours represent different animals.

protocolised changes in PEEP induced changes in ELV, and that these changes were tracked by IST, SF_6, and CT. Figure 1 and Table 2 show the comparisons of paired ELV and ΔELV measurements at each PEEP level. Statistical analyses suggested that ELV_{IST} at 0, 5, and 10 cm H_2O PEEP was not different from ELV_{SF6} or ELV_{CT} in both the uninjured lung and ARDS model. At 15 and 20 cm H_2O PEEP, the measured ELV_{IST} was lower than ELV_{SF6} and ELV_{CT} in all comparisons (Table 2).

Repeatability of ELV_{IST} measurements

The mean coefficient of variation for ELV_{IST} for each subject at each PEEP level was <5% in both the uninjured lung and the

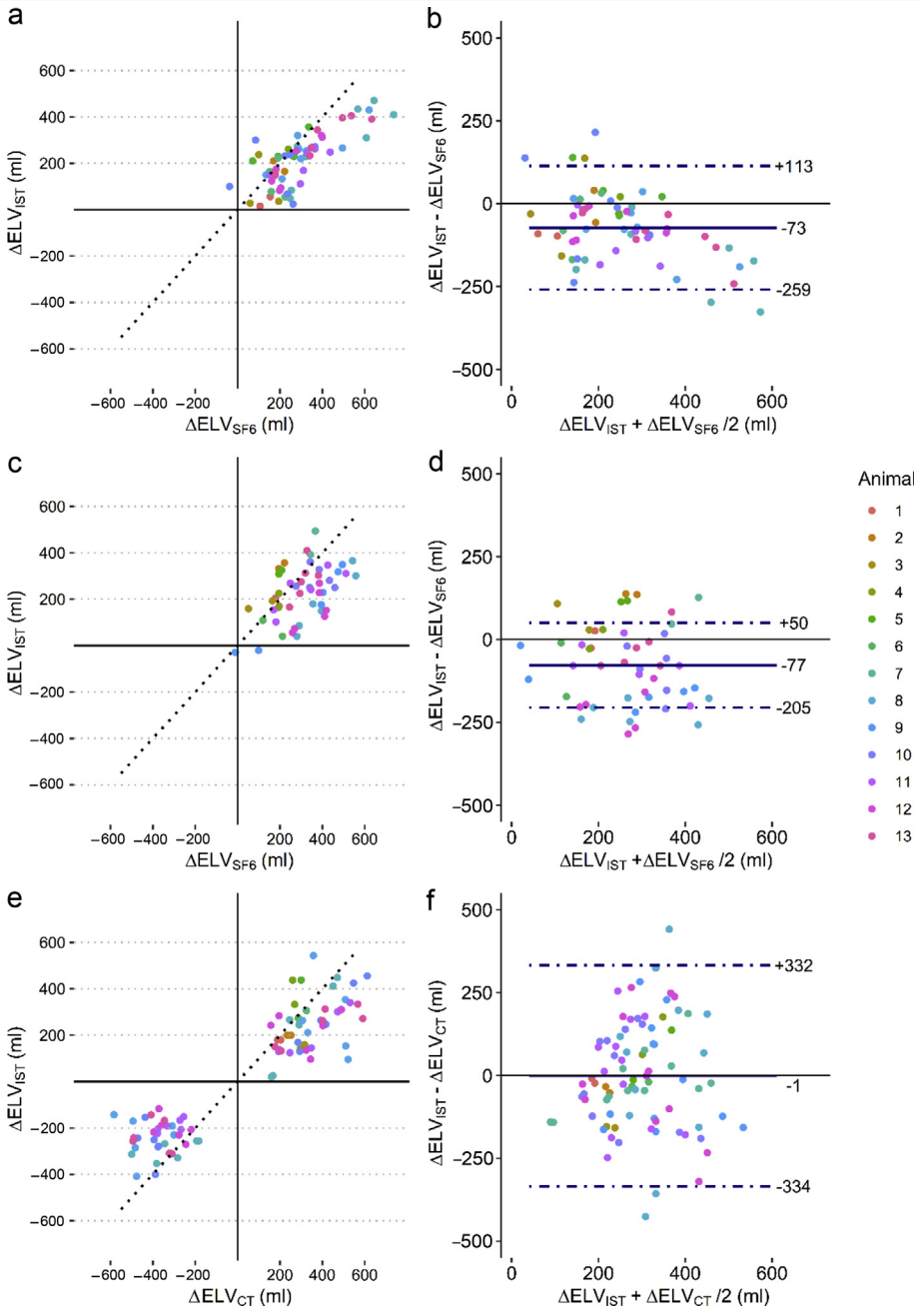


Fig 3. Scatter and Bland–Altman plots for changes in volume (ΔELV_{IST}, ΔELV_{SF6}, and ΔELV_{CT}). (a, b) Measurements from the uninjured lung. (c–f) Experiments conducted in the ARDS model. In the left column, scatterplots show changes in volume. In the right column, solid lines in the Bland–Altman plots represent the mean bias, and the dashed lines show the upper and lower limits of agreement (±1.96 standard deviation). In all plots, individual points represent a paired set of measurements, and different colours represent different animals.

ARDS model; 90% of coefficients of variation for replicate measurements were <10%.

Comparison between ELV_{IST}, ELV_{SF6}, and ELV_{CT}

Figure 2a, c, and e show scatterplots and linear mixed effects regression for each of the comparisons. There was a robust linear relationship between ELV_{IST} and ELV_{SF6} in both the uninjured lung (r^2=0.97) and the ARDS model (r^2=0.87). Similarly, we found a strong correlation between ELV_{IST} and ELV_{CT} in the ARDS model (r^2=0.92).

Agreement between ELV_{IST}, ELV_{SF6}, and ELV_{CT}

Figure 2b, d, and f show that in each comparison, ELV_{IST} has a mean bias of 12–13% less than the reference technique in both the uninjured lung and the ARDS model. ELV_{IST} has limits of agreement with ELV_{SF6} of ±17% in the uninjured lung and ±20% in the ARDS model. The limits of agreement between ELV_{IST} and ELV_{CT} were ±25% in the ARDS model.

Correlation between ΔELV_{IST}, ΔELV_{SF6}, and ΔELV_{CT}

Figure 3a, c, and e show four quadrant plots demonstrating the relationship of ΔELV_{IST} with ΔELV_{SF6} and ΔELV_{CT}. The linear relationship between ΔELV_{IST} and ΔELV_{SF6} in the uninjured lung yielded an r^2=0.58, and r^2=0.83 in the ARDS model. The linear relationship between ΔELV_{IST} and ΔELV_{CT} in the ARDS model had an r^2=0.16.

Agreement between ΔELV_{IST}, ΔELV_{SF6}, and ΔELV_{CT}

Direction of change concordance showed that ΔELV_{IST} has a concordance rate of 98–100% with ELV_{SF6} and ELV_{CT}. This is reflected in Figure 3a, c, and e, where 98–100% of data are in the top right and bottom left quadrants of each four-quadrant plot. Figure 3b, d, and f show the Bland–Altman plots with a mean bias (limits of agreement) of −73 (±186) ml for ΔELV_{IST} *vs* ΔELV_{SF6} in the uninjured lung, −77 (±128) ml for ΔELV_{IST} *vs* ΔELV_{SF6} in the ARDS model, and −1 (±333) ml for ΔELV_{IST} *vs* ΔELV_{CT} in the ARDS model. Figure 4 demonstrates the polar agreement in paired measurements. The mean angular bias ranged from 8 to 11°, and radial limits of agreement from ±30 to 37°.

Discussion

This study presents the ability of IST to measure absolute ELV and changes in ELV, compared with the 'gold standard' techniques of SF_6 wash in/washout (ELV_{SF6}) and CT (ELV_{CT}) in uninjured lungs and in an ARDS model of lung injury. In both the uninjured lungs and a saline-lavage model of ARDS, IST can reliably measure the absolute ELV comparably with SF_6 and CT, with acceptable measures of bias and limits of agreement. Additionally, when comparing PEEP-induced changes in lung volume, IST is >98% concordant with SF_6 and CT.

IST measurements of absolute ELV are below those measured by SF_6 and CT by 12–13% and do not fall within 5% of the reference method recommended by the European Respiratory Society/American Thoracic Society consensus statement.[33] These results are consistent with a previous study of IST, in which Bruce and colleagues[17] showed that IST underestimated functional residual capacity (FRC) by 32% (1080 ml) compared with body plethysmography in humans, where the mean FRC was 3.4 L. The underestimation was smaller in our study, with a mean bias of −12 to −13% (−129 to −161 ml), where the overall mean ELV was 809 ml. IST measures the volume of ventilated regions of the lung, and it is expected that IST will underestimate lung volume compared with CT, which estimates the entire thoracic gas volume. One reason for IST's underestimation of ELV compared with SF_6 may be that multi-breath washout

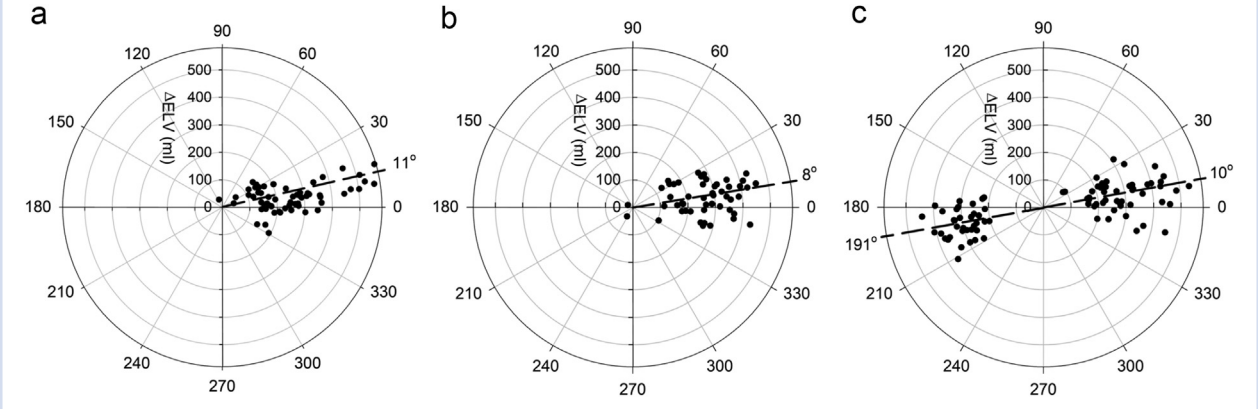


Fig 4. Polar plot analysis. The larger the mean ΔELV, the greater the distance between the point and the centre of the plot. The better the agreement between a pair of measurements, the closer the point lies to the horizontal (0°); 95% radial limits of agreement describe the angle within which 95% of the data lie. Concordance rate (%) describes the number of points that lie within the assumed limits of adequate agreement, set at ±30°. (a) Comparison of ΔELV_{IST} *vs* ΔELV_{SF6} in the uninjured lung. Mean angular bias=11°; 95% radial limits of agreement=±35°; concordance=91%. (b) Comparison of ΔELV_{IST} *vs* ΔELV_{SF6} in the acute respiratory distress syndrome (ARDS) model. Mean angular bias=8°; 95% radial limits of agreement=±37°; concordance rate 90%. (c) Comparison of ΔELV_{IST} *vs* ΔELV_{CT} in the ARDS model. Mean angular bias=10° for positive changes in volume, and 191° for negative changes in volume; 95% radial limits of agreement=±30°; concordance rate=95%.

techniques recover tracer gas from the slowest compartments if the washout period is long enough, whereas for IST, the contribution of the slowest compartment is limited by the chosen period of the sinewave.

There is no previous study assessing the ability of IST to measure changes in lung volume. The linear relationship between ΔELV_{IST} and ΔELV_{SF6} was stronger than for ΔELV_{CT}, and the limits of agreement were narrower for ΔELV_{SF6} (±186 ml in the uninjured lung and ±128 ml in the ARDS model) compared with ΔELV_{CT} (±333 ml). One proposed explanation for the narrower limits of agreement in the ARDS model compared with the uninjured model with SF_6 was that the measured ΔELV was less in the ARDS model. Further analyses showed that the measured changes in volume (based on ΔELV_{SF6}) were no different in the uninjured and ARDS models (PEEP 5–10: $P=0.11$; PEEP 10–15: $P=0.59$; PEEP 15–20: $P=0.91$). The repeatability of IST measurements of ELV in the same subject under the same conditions was within the reported international acceptable limits of coefficient of variation (<10%)[34] and consistent with studies of IST in other circumstances.[17] IST has good repeatability and minimal bias compared with CT (mean bias=−1 ml) when measuring changes in lung volume. This provides support for the use of IST to monitor trends in lung volume, where the change (or lack of) in volume after a change in ventilator setting is potentially of most interest to the bedside clinician.

The inspired sinewave technique offers several strengths, including commercial availability of component parts and its ease of use at the bedside. As IST does not rely on a fixed inspiratory flow, it can be used in spontaneously ventilating patients. The main limitations of our study are that the use of a porcine model does not directly translate to humans, and the use of saline lavage does not necessarily comprise all the features of human ARDS. However, saline lavage does result in reduction in lung volume, which is one reason for its choice in this study. In addition, in this protocol, not all PEEP levels were studied in all experiments. This was not feasible due to time restraints because the initial seven experiments including PEEP 0, 5, and 10 cm H_2O were undertaken in parallel with data collection for another study, in keeping with the 3Rs principle.[35] Future development of IST could include assessing the feasibility of its use in mechanically ventilated human patients. IST development will enable the measurement of lung volumes when using volume-controlled and pressure-controlled ventilation modes in paralysed patients and in assisted mechanical ventilation, where less regular inspiratory flow patterns exist.

The inspired sinewave technique is a potentially translatable research tool and this study is the first to examine its use in mechanically ventilated lungs. Measuring the size of the ARDS baby lung would allow titration of ΔP to the individual patient, either directly[7] or by scaling V_T to the size of the baby lung. This has the potential to reduce mortality in patients with ARDS. We have demonstrated that IST measured lung volumes and changes in volume reliably compared to the standard techniques of SF_6 washin-washout and CT in a mechanically ventilated porcine model. Our results support further development of this technique and its translation to human medicine.

Authors' contributions

Study design: DCC, FF, PAP
Experimentation: DCC, JNC, MCT, PAP
Data analysis: DCC
Data interpretation: DCC, MCT, FF, JNC, ADF
Financial support: FF, PAP, AL, ADF
Writing manuscript: DCC
Critical revision: DCC, MCT, JNC, FF, AL, ADF

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Declarations of interest

ADF and PAP are named inventors on a patent application (EP3122249A1/US20170100043A1 [pending]) submitted by the University of Oxford, which may receive financial benefit when the technology is commercialised.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bja.2019.11.030.

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