Assessment Of Compliance In An Experimental Setting

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1. Background

Assessing and reassessing quality of donor lung is an essential step before lung transplantation[1, 2]. However， which parameters should be included in the assessment is still in debate[1, 3, 4]. Physiological measurements such as pulmonary arterial oxygen saturation and respiratory compliance can be relatively easily explored in vivo and ex vivo and are used routinely in lung assessment before and after lung transplantation as well as to assess the effect of reconditioning techniques such as ex vivo lung perfusion (EVLP)[2, 5].

1.1 Respiratory compliance

Respiratory compliance represents the ratio between the volume of air present in the lungs and the related pressure. It is the resultant of chest wall compliance and lung compliance. The relationship between thoracic and lung compliance is the following:

$$\frac{1}{Respiratory\ compliance} = \frac{1}{thoracic\ compliance} + \frac{1}{lung\ compliance} \quad (1)$$

Thoracic compliance is ascribable to the thoracic cage structures' (sternum, ribs and respiratory muscles) mechanical properties. Evidently, structural defaults of the chest wall alter thoracic compliance i.e. patient suffering from thoracic osteoarthritis have stiffer thoracic cages, hence lower thoracic compliance than average. Lung compliance is related to the lung parenchyma itself and is thus conceptually more pertinent in physiological studies[6]. Even if lung compliance is of major interest, measuring it in vivo requires the measurement of esophageal pressure, a surrogate of pleural pressure. Due to technical complexity this is not performed as standard measurement and practically only respiratory compliance[7] is usually measured.

1.2 Measurement of respiratory compliance

Several techniques are available to measure respiratory compliance in the experimental setting. We can cite, on one hand, dynamic compliance measurement, which is usually computed as the ratio between tidal volume and maximal airway pressure during volume controlled ventilation[8]. On another hand we have more reliable static or quasi-static measurements as automated pressure-volume (PV) curve, semi-automated measurement based on plateau pressure determination and manual measurement using a manometer[9].

In our study, we would like to focus on the 3 static and quasi-static measurements of respiratory compliance mentioned above.

1.2.1 Automated measurement of compliance

The automated compliance measurement technique performed by the ventilator used in our experiments is similar to the basic super-syringe technique[10, 11]. From the end expiratory volume, the ventilator insufflates air and sequentially stops at known tidal volumes (steps of 0.5 ml). Between each step a pause of 1 second is permitted to reach stable state with zero flow. The machine performs airway pressure measurement during this period. Based on the volumes injected and on the sequential pressure measurements performed, a P-V curve is automatically displayed. (See Fig. 1.1) The static compliance is determined by measuring the P-V curve slope.

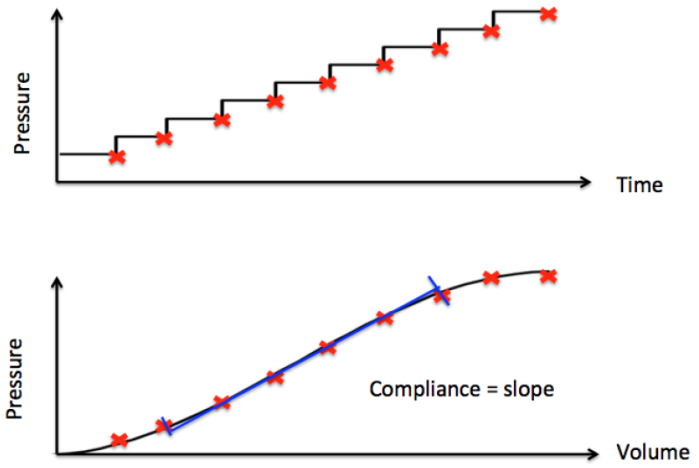

Fig. 1.1 The ventilator injects a tidal volume in multiple steps of 0.5ml each time (graph above) in order to complete P-V curve (graph below) and calculate the slope, which is equals to static compliance.

1.2.2 Semi-automated measurement of compliance

The semi-automated measurement technique is based on plateau pressure (P_{plat}) and total end-expiratory pressure measurements and on the equation of motion of the respiratory system[11].

The respiratory system equation of motion describes the relation between lung volume, airway pressure and compliance. In a dynamic model, the airflow, airway resistance and impedance play non-negligible roles in the airway pressure. During controlled ventilation, in the absence of spontaneous breathing, the equation of motion of the respiratory system is written as follow:

$$P_{aw}=\frac{V}{C}+V^{\circ}R+PEEPt \quad (2)$$

where P_{aw} is the airway pressure, V is the lung volume, C is the respiratory system compliance, V° is the inspiratory flow, R is the airway resistance, $PEEP_t$ is the total positive end-expiration pressure.

If we consider a static model (for example during an inspiratory pause) where airflow, airway resistance and impedance are zero, we can simplify this equation to:

$$P_{aw}=\frac{V}{C}+PEEPt \quad (3)$$

The semi-automated measurement is performed as follows. $PEEP_t$ is measured by performing an end-expiratory occlusion[12]. At the end of inspiration, an inspiratory pause is then performed to measure P_{plat}, which corresponds to alveolar pressure (See Fig.1.2). Using measured $PEEP_t$ and P_{plat} and the known inspiratory tidal volume, compliance can be calculated using the previously mentioned formula as:

$$Compliance = \frac{Vt}{Pplat-PEEPt} \quad (4)$$

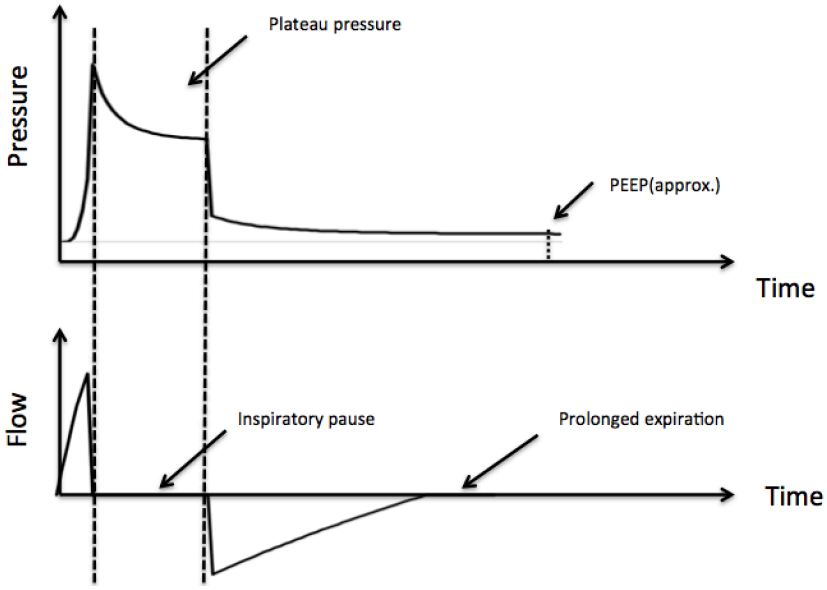

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Fig. 1.2 A complete measurement cycle using the semi-automated technique. By creating an inspiratory pause and a prolonged expiration, plateau pressure and approximate PEEP were measured.

To be noted the semi-automated technique does not require high inflation pressure and can thus be repeated several times without damaging the lungs or creating hemodynamic instabilities, which is a clear advantage in clinical setting[13].

1.2.3 Manual measurement of compliance

The principle of the manual technique is to use the basic principles of C=ΔVolume/ΔPressure (Figure 1.3), most employed technique in laboratory[11, 14]. For this technique the ventilator is disconnected and the artificial airway inserted into the rat trachea is connected to a three-way stock, itself connected to a syringe and a manometer. The baseline pressure (empty lungs) is measured by connecting the monometer to the artificial airway. An inflation volume is then injected in the lungs

using the syringe and the inflated lungs pressure is measured using the manometer. Compliance is computed as C = injected volume/ (inflated lung pressure – empty lung pressure).

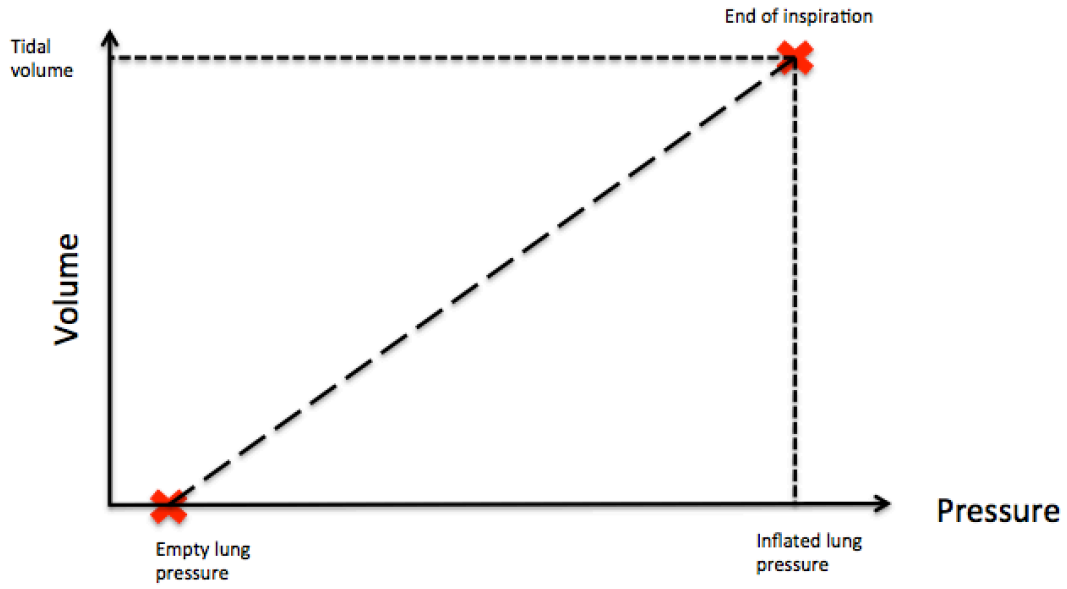

Figure 1.3 Manual measurement of compliance with determination of inflated lungs pressure and empty lungs pressure with a known inflation volume

2. Aim of the study

Concerning respiratory compliance determination, as previously described, several methods are available. In the experimental setting of rat lung transplantation, the concordance of the results obtained with the various available techniques is unknown[3]. The main aim of our study is to compare the commonly used lung compliance measurement techniques.

The effect of sternotomy on compliance has not been extensively described in rat. Our secondary aim is to assess the effect of sternotomy on lung compliance in the experimental setting of rat.

As a last aim, we would like to compare effect of EVLP on lung compliance using the automated technique.

3. Methods

3. 1. Experiment design

We conducted an experimental study in 8 rats to compare the differences between automated, semi-automated with plateau pressure determination and manual techniques for measuring static pulmonary compliance in vivo before and after sternotomy.

In 2 other rats, we assessed the EVLP treatment on lung compliance. We also measured compliance using the automated and manual techniques in vivo before and after sternotomy and following the ELVP protocol detailed by Wang et al.[15]. At 30 min and 60 min of EVLP perfusion we measured compliance respectively using the automated technique again.

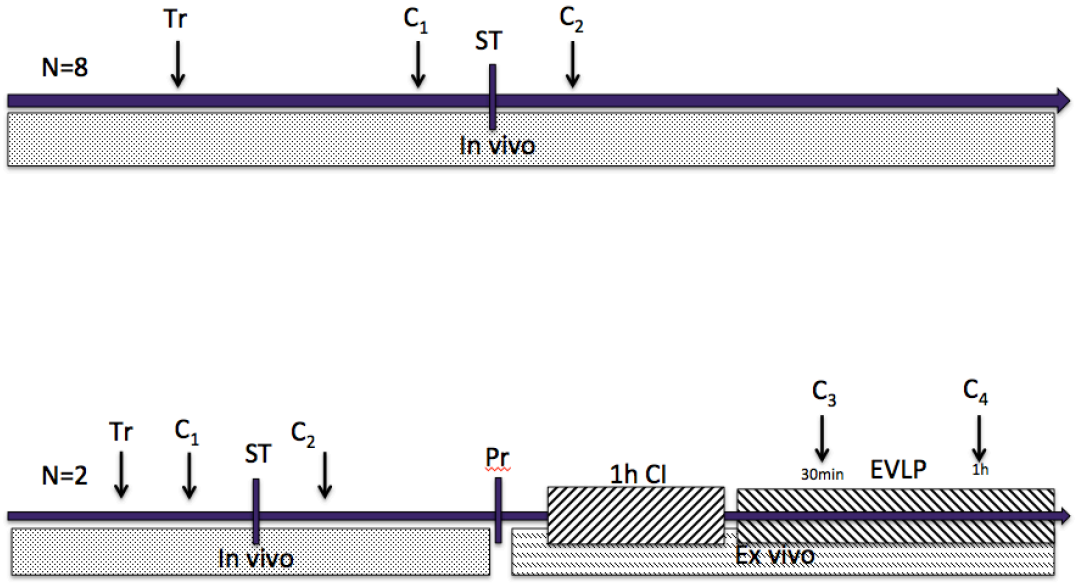

Figure 3.1 Tr=Tracheotomy and intubation; C_1-C_4= Compliance measurement; ST=Sternotomy; In vivo= Lung before Pentobarbital overdose and exsanguination; Ex vivo= Lung after Pentobarbital overdose and exsanguination; Pr= Procurement; CI= Cold ischemia; EVLP= Ex vivo lung perfusion

3.2. Materials

8 male adult Sprague-Dawley rats (10-14 weeks; weight between 342-422g, Charles River, L'Arbresle, France) were used for techniques comparison study. 2 male adult Sprangue-Dawley rats (14-16 weeks, weight 465 and 496) were used for EVLP treatment study. The experiment was approved by the ethical committee (authorization 2637), under the program for lung transplantation experiment.

3.3. Experiment description

In the non-EVLP group of 8 rats, 250 units of heparin were injected

intraperitoneally 10 minutes before the experiment started to prevent thrombus due to surgical procedures. Anesthesia was induced with 5% Isoflurane inhalation, and maintained with intraperiotoneal pentobarbital sodium injection (50mg/kg body weight). The subjects were placed on a custom heat plate to maintain body temperature at 37.5°C. We also monitored animals' oxygen saturation and arterial pressure with a rodent pulse oximeter (Starr Life Science Corp. Oakmont, USA) and a patient monitor (Datex-Ohmeda, Inc. Madison, USA). Animals were perfused through femoral vein with 5% NaCl solution to refill lost volume and to maintain an adequate arterial pressure.

The animals were tracheotomized and a 16gauge metallic cannula was inserted in their trachea. The cannula was advanced to 0.5cm of the carina and tightened with a 3-0 silk suture and connected to a Flexivent FX3 ventilator (SCIREQ Inc, Montréal, Canada). The animals were then mechanically ventilated (respiratory rate 80min^{-1}, tidal volume 7$mlkg^{-1}$, positive end of expiration pressure 3mlH_2O, fraction of inspired oxygen 21%).

After 7 minutes of mechanical ventilation the ventilation was paused and three recruitment maneuvers with sequentially 12cm H_2O, 15cm H_2O and 18cm H_2O were made. Immediately after the third recruitment maneuver, we started the measurement of compliance first using the automated technique. The ventilator stopped the normal ventilation cycle and injected a calibrated tidal volume in several steps of 0.5ml each time. The change in volume and pressure resulted are registered by the FlexiVent system (SCIREQ Inc, Montréal, Canada)（Figure 3.2). The P-V curve is plotted and slope of the curve is calculated automatically (Figure 1.1).

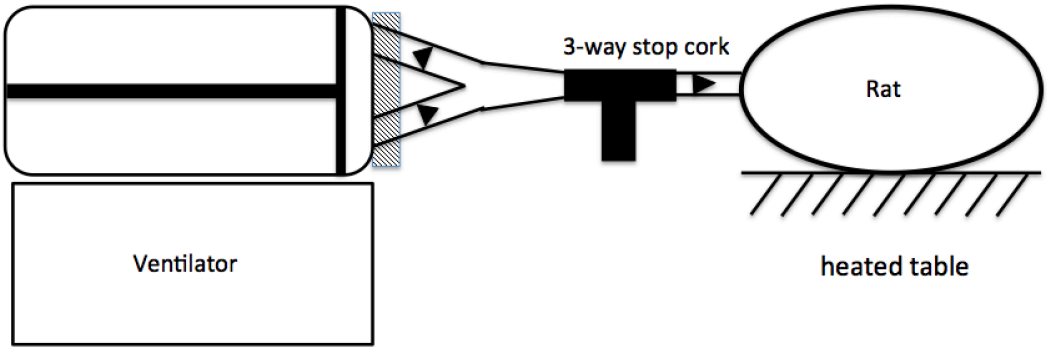

Figure 3.2 Automated technique setting, where ventilator injected a tidal volume in multiple steps of 0.5ml each time and measured the change in airway pressure resulted. By drawing the P-V curve, compliance is determined.

Before beginning measurement of compliance with the semi-automated technique, we connected the 3-way stop cork to the MP-100 system (BIOPAC Systems,Inc. CA, USA) to monitor the pressure continuously (Figure 3.3) and adapted

the ventilation pattern to increase expiratory time (respiratory rate 20min^{-1}, tidal volume 7mlkg^{-1}, positive end of expiration pressure 3mlH_2O, fraction of inspired oxygen 21%) in order to measure positive pressure at the end of expiration (PEEP). This measured PEEP is considered as a good approximation of $PEEP_t$. The $PEEP_t$ should theoretically be measured with an expiratory occlusion [16], which is not possible with the ventilator we used for this experiment. At the end of inspiration, we performed an inspiratory pause to measure P_{plat} (Figure 3.4), which corresponded to alveolar pressure (Figure 1.2). Using measured approximated $PEEP_t$, P_{plat} and the known inflation tidal volume, compliance can be calculated using the previously mentioned formula：

$$Compliance = \frac{Vt}{Pplat - PEEPt} \quad (4)$$

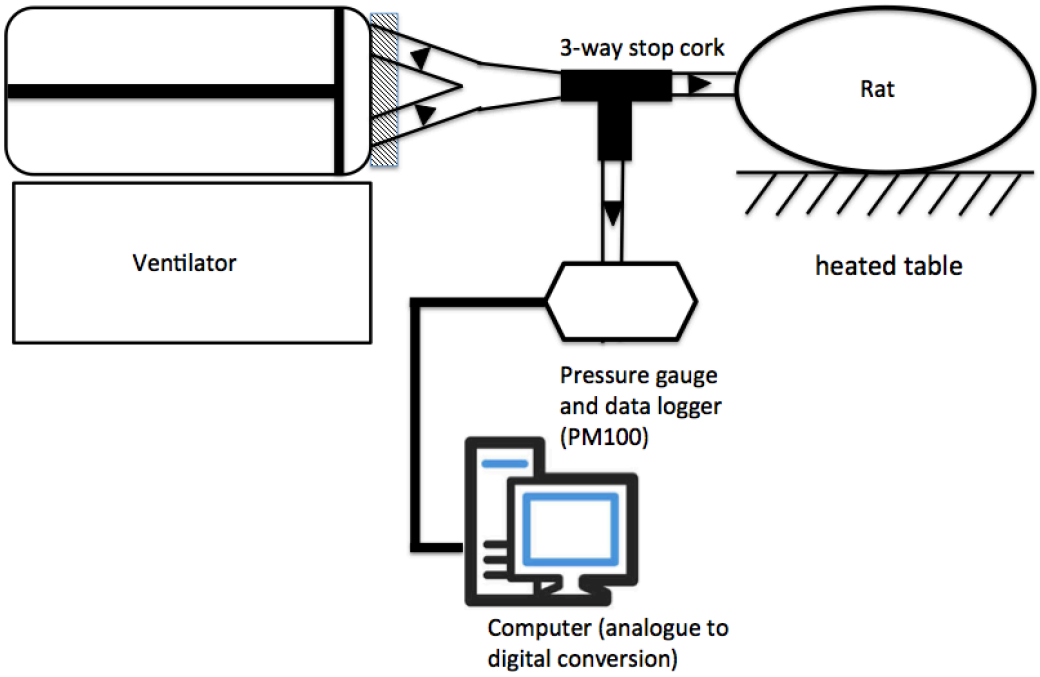

Figure 3.3 Semi-automated technique setting

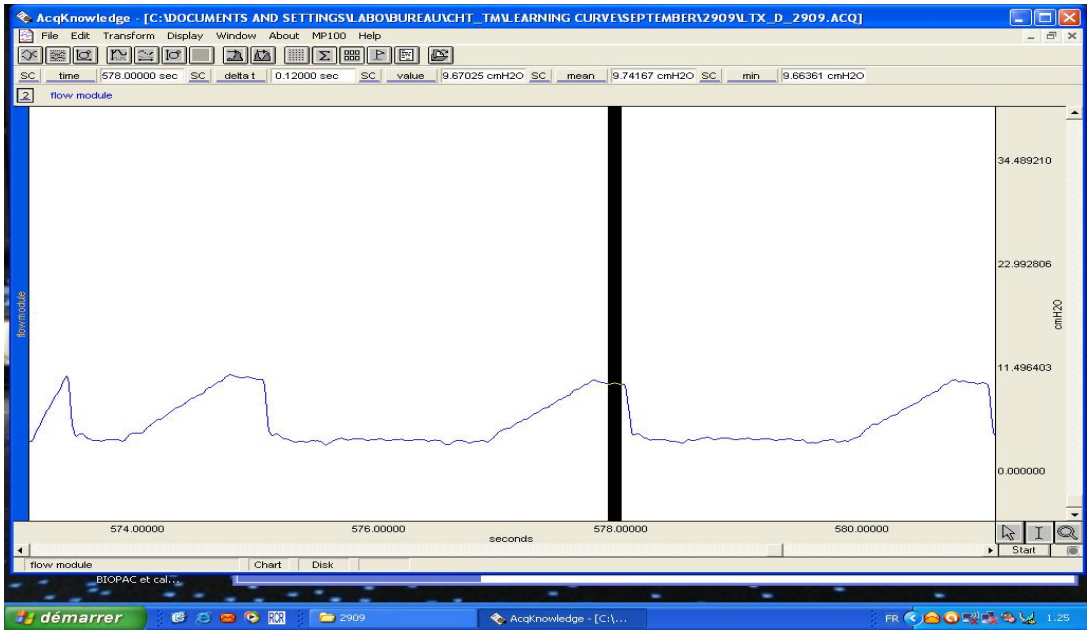

Figure 3.4 Screen shot of monitoring and determination of P_{plat} with the semi-automated technique

For the manual technique we disconnected the ventilator completely from the rat at end of expiration. 3-way stop cork is closed to maintain the lungs in functional residual volume. Then we connected a syringe to the 3-way stop cork and injected a tidal volume in the lungs. The airway pressure is measured using a manometer connected to the other side of the 3-way stop cork. The pressure at end of the tidal volume reflects a full lung expansion pressure, which is a good estimation of P_{plat}. We then allowed a spontaneous expiration by removing the syringe. The pressure at the end of the expiration is a good estimation of $PEEP_t$ (Figure1.3). Applying again formula (4), we calculated the compliance.

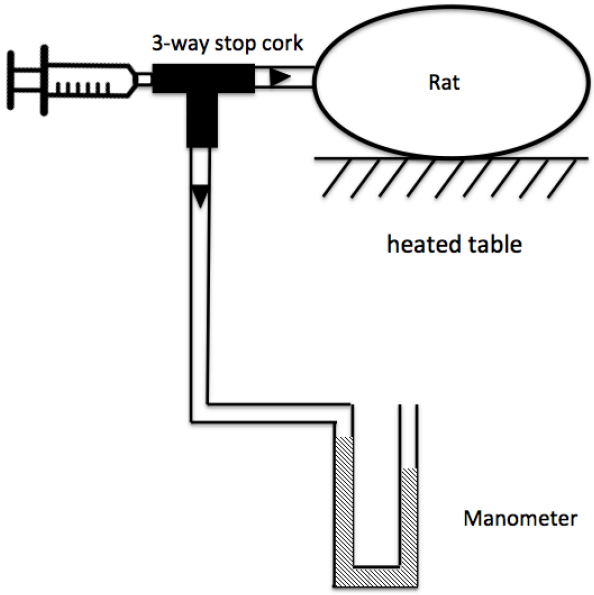

Figure 3.5 Manual technique setting

Both semi-automated and manual measurements are repeated at least once. Between each technique, another recruitment maneuver was performed with 12cm H_2O to grant alveoli recruitment. Shortly after the last compliance measurement, the animals were sternotomized without dissecting the lungs from the thorax. The static compliance was measured again using the 3 techniques, also with 12cm H_2O recruitment maneuver in between.

In the EVLP group of 2 rats, the initial procedures were the same as the non-EVLP group, where static compliance is measured using automated and manual techniques before and after sternotomy, but not with semi-automated technique. Once all the measurements were collected, animals were killed humainly with Pentobarbital overdose. We then clamped and cut the aorta and vena cava and immediately flushed the heart and lungs with Perfadex (Xvivo Perfusion, Götenborg, Sweden) through perfusion cannualae (Hugh Sachs, Hugstetten, Germany) inserted in the pulmonary artery and left atrium. The lungs are inflated (FiO_2 =0.21) and preserved in 4 °C Perfadex for an hour. The heart-lung block was weighed and exposed to an hour of EVLP using a customized rat EVLP system (Harvard IL-2 System, Hugo Sachs Elektronik & Harvard Apparatus, March, Germany). After 30 minutes of ex vivo perfusion we measured the static compliance using the automated technique. After 1 hour of ex vivo perfusion the static compliance is measured again using the automated technique. (Figure1.1)

3.4. Statistic analysis

All the measurements are represented in table 3.1. All the data are expressed as mean±SD. We took the average of two repeated measures from semi-automated and manual techniques to compute the weighted result. Due to practical difficulties for instance when the lungs are in venerable state, only one of the two measurements for the semi-automated and manual measurement was made. In this case, we used the one value available to compile the result table. We tested normality of the results with the D'Agostino & Pearson omnibus normality test. Since all results were parametric, we then used the parametric 1way analysis of variance for repeated measurements to compare the different techniques, with Bonferroni correction. To compare the mean difference between techniques, Bland-Altman tests are performed between each two techniques. Effect of sternotomy was analyzed with a paired t-test, and each sub-group of different technique is analyzed again with a paired t-test. Chi^2 test was used to assess the distribution of variance among techniques. We performed a 2way analysis of variance test to evaluate the interacation effect, corrected with the Tukey correction. P less than 0.05 were considered significant. Data analyses were performed by Graphpad Prism 6 (GraphPad Software Inc., La Jolla, USA) and MedCalc (MedCalc, Ostend, Belgium).

no. rat	Automated measurement		Semi-automated measurement		Manaul		EVLP 30 min	EVLP 60 min
	BS	AS	BS	AS	BS	AS		
1	1	1	2	2	2	2	0	0
2	1	1	2	2	2	2	0	0
3	1	1	1	1	2	2	0	0
4	1	1	2	2	2	2	0	0
5	1	1	2	2	2	2	0	0
6	1	1	2	2	2	2	0	0
7	1	1	2	2	2	2	0	0
8	1	1	2	2	2	1	0	0
9	1	1	0	0	2	2	1	1
10	1	1	0	0	2	2	1	1

Table3.1 Table of number of measurement, 0 means unindicated or not planned.The two subjects which underwent EVLP treatment (number 9 and 10) are included in statistic tests due to limited number , however the general trend is illustrated (Figure 4.10).

4. Results

4.1. Comparison of 3 techniques of measurement

All results from the 3 groups were illustrated in Figure 4.1. When comparing the difference of median compliance measured using the 3 techniques, we see a significant difference($p<0.01$; Figure 4.1). In the test between subgroups, there was a significant difference between automated and semi-automated techniques ($p<0.0001$); a significant difference between manual and semi-automated techniques ($p<0.0001$); no signficant difference was observed between automated and manual techniques ($p=0.4222$). The coefficients of variation for automated semi-auto and manual groups are 14.43%, 17.55% and 13.97% respectively. We see that automated technique yields highest average compliance and semi-automated technique yields lowest average compliance.

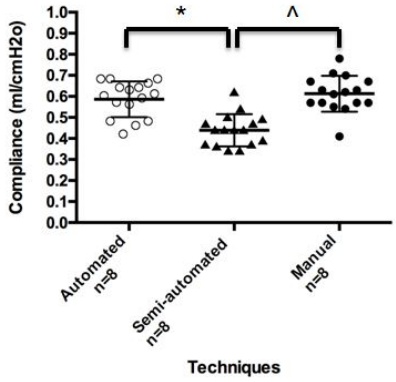

Figure 4.1 Comparison of 3 techniques regardless of sternotomy, n=number of animals, with two point for each animal with each technique, representing before and after sternotomy. *P<0.05 vs Semi-automated; ^P<0.05 vs Semi-automated.

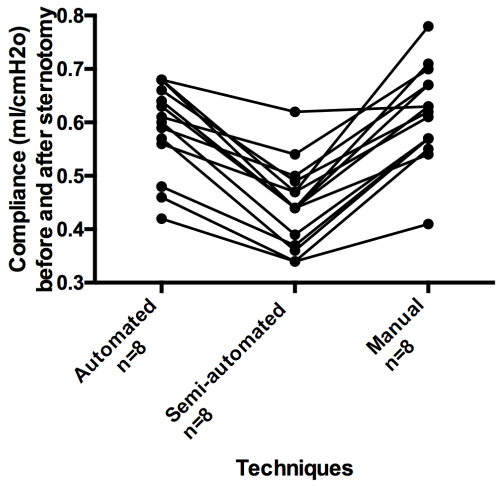

Figure 4.2 Visual representation of compliance of each animal. n= number of animals. For each animal two measurements (before sternotomy and after sternotomy) are made (16 values for each technique). Lines connect compliance with different techniques from the same animal under same condition.

As illustrated in the Bland and Altmann plots below the three methods of compliance measurement are relatively well correlated. When the semi-automatic and automatic measurements were compared (Figure 4.3a), the bias was – 0.15 ml/cmH_2O with lower and upper limits of agreements of respectively -0.27 and -0.03 ml/cmH_2O. This suggests that values obtained with the semi-automatic techniques are globally lower than values obtained with the automatic technique.

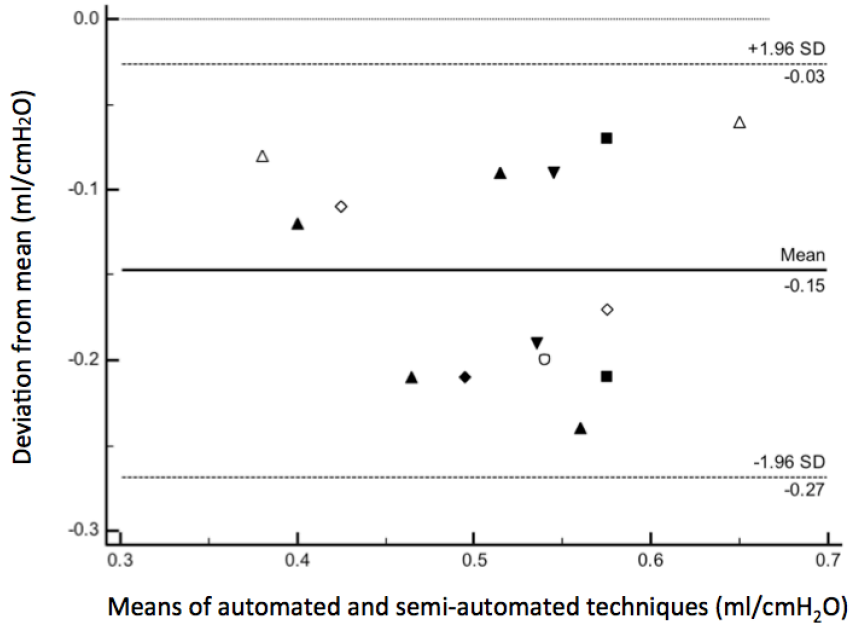

Figure 4.3a Bland-Altmann plot of automated and semi-automated techniques with semi-automated technique designated as the reference.

When the semi-automatic and manual techniques were compared (Figure 4.3b), the bias was -0.17 ml/cmH_2O with lower and upper limits of agreements of respectively -0.32 and -0.03 ml/cmH_2O which also indicated that the semi-automatic technique provided lower values.

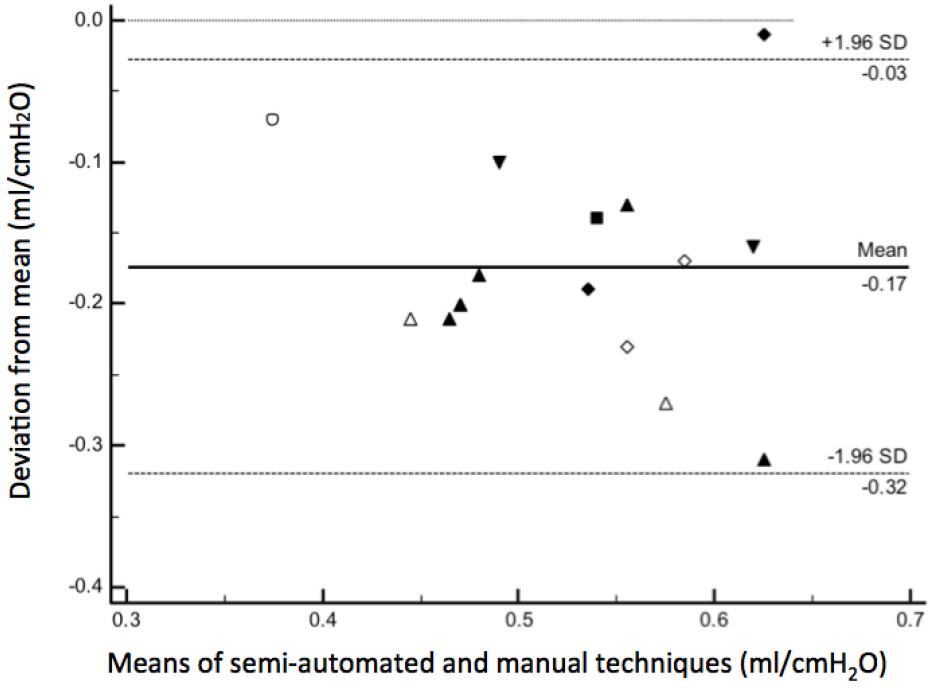

Figure 4.3b Bland-Altmann plot of semi-automated and manual techniques with semi-automated technique designated as the

reference.

When the automatic measurement technique was compared with the manual technique (Figure 4.3c), the bias was – 0.03 ml/cmH_2O with lower and upper limits of agreements of respectively – 0.16 and 0.10 ml/cmH_2O, meaning that both techniques gave relatively similar results.

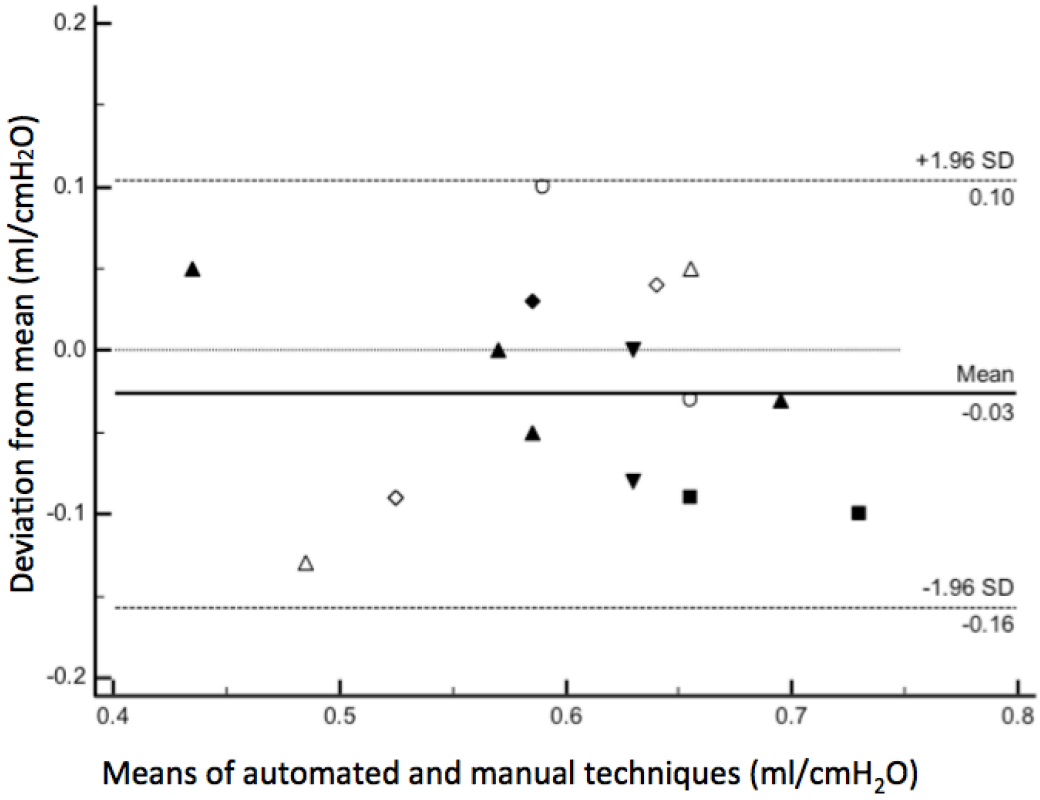

Figure 4.3c Bland-Altmann plot of semi-automated and manual techniques with manual technique designated as the reference.

4.2.Effet of sternotomy

Sternotomy increase compliance in all groups combined ($p<0.01$, Figure 4.4a), by an average of 0.071ml/cmH_2O. Using Pearson's Chi^2 test we see the coefficient of variance of sternotomy was not significant for the 3 techniques ($p=0.47$).

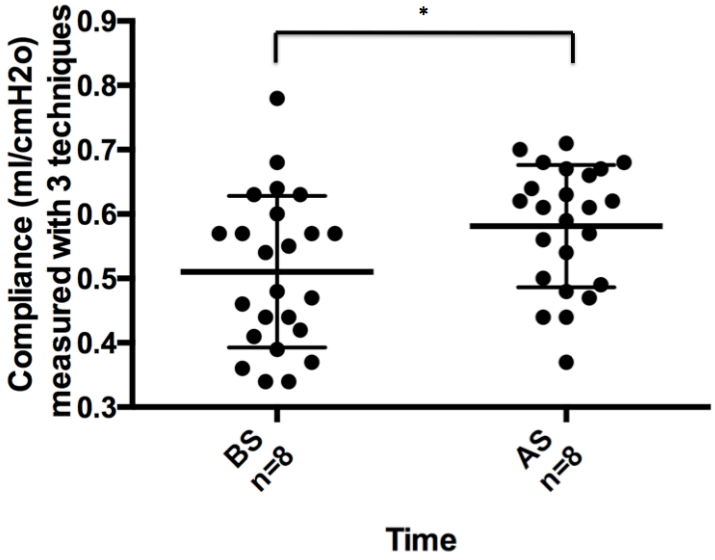

Figure 4.4a Effect of sternotomy assessed by the 3 techniques, n= number of animals, for each animal 3 value for each animal at each time point. BS= before sternotomy, AS= after sternotomy; *P<0.05 vs BS.

In the automated sub-group, no significant difference is observed (p=0.2958, Figure 4.4b)

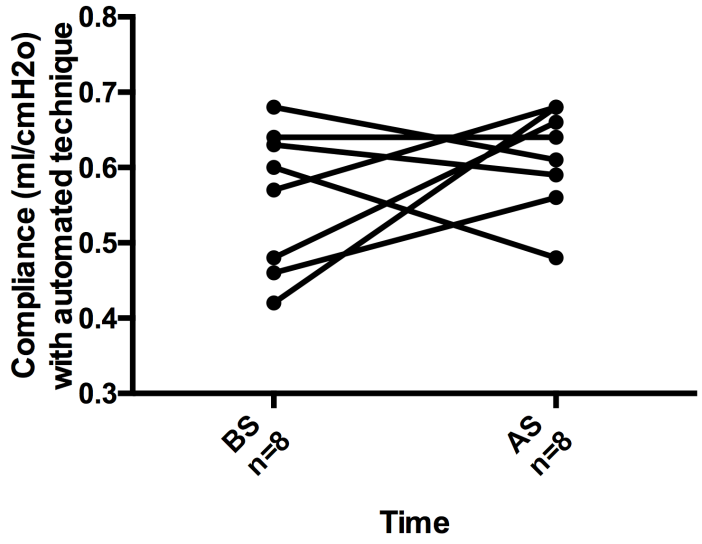

Figure 4.4b effect of sternotomy on measurement of compliance with automated technique (p=0.2958), n= number of animals BS= before sternotomy, AS= after sternotomy.

In the semi-automated sub-group, effect of sternotomy is significant (p<0.05, Figure 4.4c).

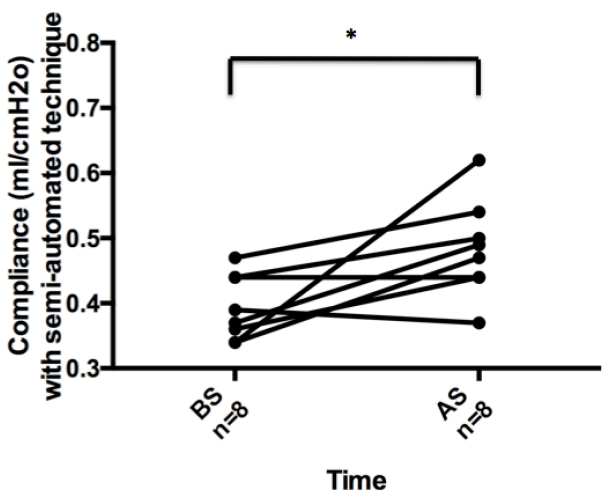

Figure 4.4c effect of sternotomy on compliance measured with semi-automated technique (*P<0.05 AS vs BS). n= number of animals, BS= before sternotomy, AS= after sternotomy.

In the manual sub-group, effect of sternotomy is not significant (p=0.0592, Figure 4.4d).

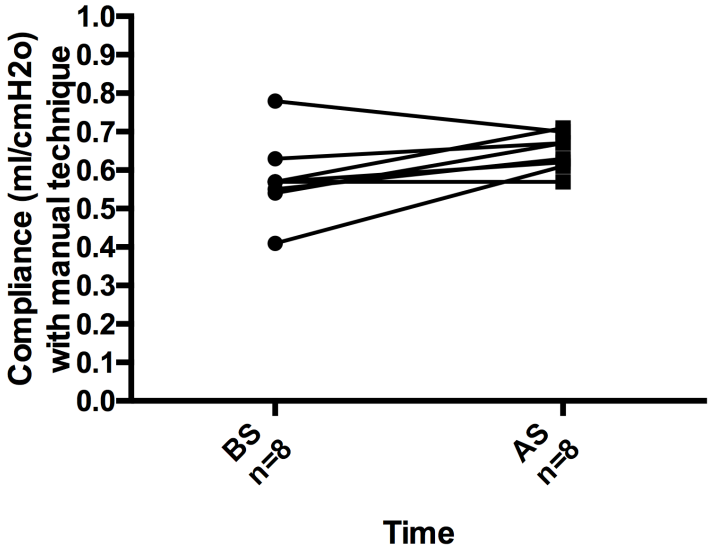

Figure 4.4d effect of sternotomy on manual technique (p=0.0592), n= number of animals, BS= before sternotomy, AS= after sternotomy.

4.3 Comparison of interactions

When assessing the crossed effects of techniques and sternotomy, the interaction effect is negligible (p=0.4977), effect of sternotomy is significant (p<0.01), effect of technique is significant (p<0.01).

4.4 Effect of weight

When comparing the effect of weight on automated compliance, there is no significant correlation between weight and compliance, for both before and after sternotomy(p=0.7685 and p=0.5135 respectively, Figure 4.5a).

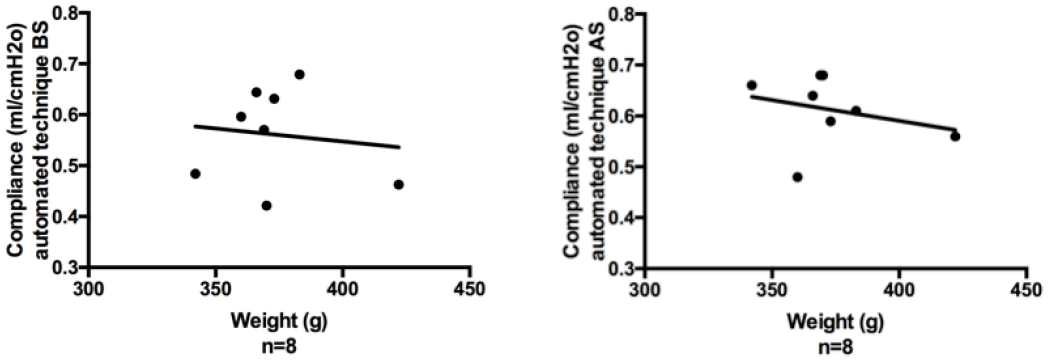

Figure 4.5a Comparison of compliance measured with the automated technique classed by body weight before and after sterntomy. (BS p=0.7685; AS p=0.5135) n=number of animals, BS= before sterntomy, AS= after sternotomy.

When comparing the effect of weight on semi-automated compliance, there is no significant correlation between weight and compliance, for both before and after sternotomy(p=0.7446 and p=0.8070 respectively, Figure 4.5b).

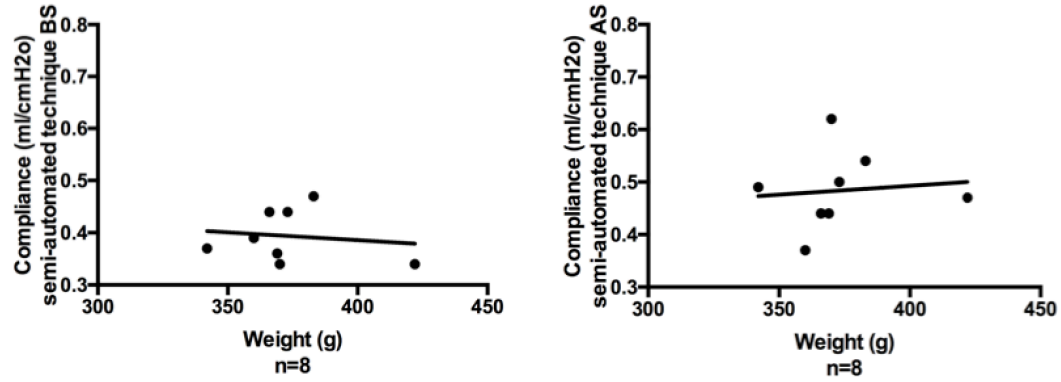

Figure 4.5b Comparison of compliance measured with the semi-automated technique classed by body weight before and after sterntomy. (BS p=0.7446; AS p=0.8070) n=number of animals, BS= before sterntomy, AS= after sternotomy.

When comparing the effect of weight on manual compliance, there is no significant correlation between weight and compliance, for both before and after sternotomy(p=0.4240 and p=0.9534 respectively, Figure4.5c).

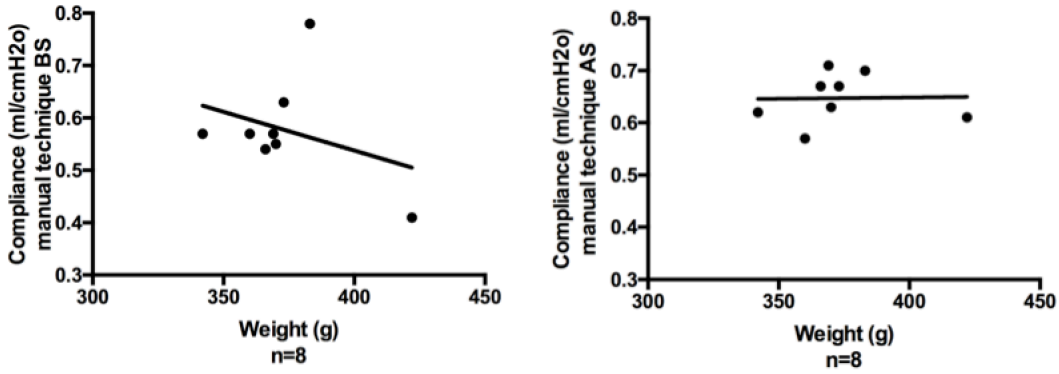

Figure 4.5c Comparison of compliance measured with the manual technique classed by body weight before and after sterntomy. (BS p=0.4240; AS p=0.9534) n=number of animals, BS= before sterntomy, AS= after sternotomy.

4.5Compliance measured using the automated technique with EVLP

In the EVLP treatment group the compliance measured is consist with the non-EVLP group. After 30 minutes of perfusion rewarming and 10 minutes of ex vivo ventilation the compliance has a marked decrease. After 40 minutes of ex vivo ventilation the compliance increased, without obvious oedema formation on the lungs.（Figure 4.6)

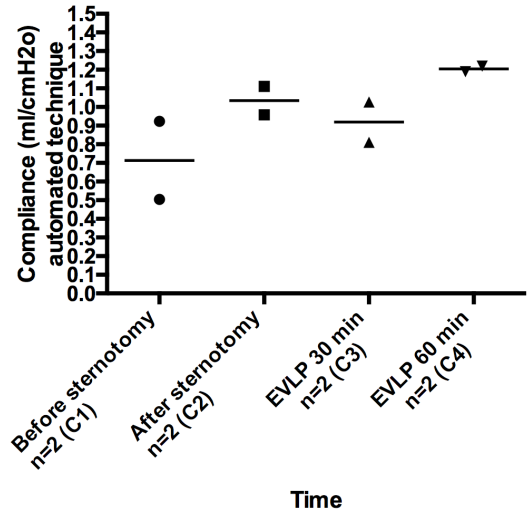

Figure 4.6 Comparison of compliance measured using automated technique before and during EVLP, n=number of animals C_1-C_4 correspond to different time points mentioned in figure 3.1.

5. Discussion

Our study demonstrated that all 3 techniques are valid techniques for compliance measurement, with good precision.

By convention the automated technique is the standard technique of measuring compliance, with the advantage of feasibility and less manipulation errors, oppositely to the semi-automated technique, which is cumbersome with the equipment necessary for measuring and post hoc analysis with Acquire®, increasing its difficulty in application. However our study clearly illustrated that unfortunately automated technique was not sensitive to the change in compliance due to sternotomy, hence shadows a doubt onto its precision in the EVLP study, where it is the only technique usable for compliance measurement.

We confirm that sternotomy does increase lungs compliance in a significant way. This is probably due to removal chest wall restriction on the lungs, which allows a better lung expansion. Semi-automated technique yielded the lowest values of 3 techniques and was the only one that illustrated the effect of sternotomy on compliance. In the automated and manual technique groups we did not observe a significant increase of compliance after sternotomy, due to possibly the power effect of a small number of subjects, as observed in Figure 4.4 and 4.6. Given a sufficiently large population we would probably rule out the effect of outliner. Nevertheless the outliner in the automated group was not the same outliner in the manual group.

We hypothesize that the narrow airway and possible bronchial secretion in rat influence compliance in a significant way[17]. In clinical setting a flow of 3l/min is considered as a low flow model[18], in our experiment we have adapted the flow to 0.018l/min to readjust for the small size of rats, however is 0.018 l/min a low flow for rat remains a question to be answered.

Charles et al.[19] have cited that in human pulmonary compliance is related to functional residual capacity, which in turn is related to an exponential function of body weight. Yet in humans the relationship between chest wall compliance and body weight is not clear[20, 21]. We thought weight to be an intrinsic factor influencing the compliance in rat. The heavier the bodyweight the greater chest wall restriction would become, hence less compliant the lungs are. Hence sternotomy in heavier rats would result in a greater increase proportionally in rats. However such a relationship was not observed in our study, suggesting in rats the weight is not a good indicator to

compliance.

We consider in parallel height as an indicator to lung size, as observed in human. Could this also be true in rats? In laboratory the size of the rat is not usually measured, given that the tail can be as long as the body (20-25cm for body and 18-25cm for tail). Could we measure the rat by their size without the tail rather than weight to have a better correlation with their lung size would be due to further research in the field.

For the EVLP group we can see a global increase of compliance with EVLP. During ex vivo perfusion, edema formation was reduced with a stable hypertonic perfusion and rewarming, thus improving hemodynamic profile of the lungs. This is no significant difference between compliance measured after sternotomy compared to after 10 minutes of EVLP ventilation, suggesting EVLP is a slow process. Kuiper et al. [22] have observed septic insult decrease lung oxygenation and compliance and that mechanical ventilation alone would not improve either of these two parameters. Hence indicating the perfusion facet of EVLP may be a crucial in lung reconditioning. If we would like to truly assess respiratory physiology of EVLP in rats, larger sample experiments have to be conducted.

We see several possible source of error in the experiment. For instance during semi-automated measurement the rats have had several spontaneous breathings, which interfered with the measurement.

Better codification of the experiment protocole has to be defined and implemented. We have observed a correlation between higher recruitment pressure and greater compliance, due to probably less atelectasis formation. However we observe obvious damage to lung tissue post sterntomy with recruitment pressure higher than 18 cmH_2O.

In addition we have always performed the measurements by the order of automated, semi-automated and manual techniques. There may be parenchymal over extension due to repeated measures, hence randomized sequenced measurement experiment would be advised. The measurements done after sternotomy would have also been affected by the measurements made before sternotomy, hence ideally we would need two groups of rats in one only before sternotomy compliance has been measured and in the other only after sternotomy.

6. Conclusion

We conclude the 3 different techniques are concordant but with different intrinsic variabilities. Sternotomy increases compliance in a significant way. EVLP

treatment improves lung condition and increases compliance after cold ischemia in rats.

We see compliance as an important parameter and useful indicator of lung assessment. Ho can we fully explore the potential of lung compliance and unify measurement techniques remain questions to be answered with further investigations.

Annex

Table of results

no. rat	Weight (g)	Automated measurement		Semi-automated measurement		Manaul		EVLP 30 min	EVLP 60 min
		BS	AS	BS	AS	BS	AS		
1	366	0.64	0.54	0.44	0.44	0.54	0.67		
2	373	0.63	0.59	0.44	0.5	0.63	0.67		
3	370	0.42	0.68	0.34	0.62	0.55	0.63		
4	369	0.57	0.68	0.36	0.44	0.57	0.71		
5	383	0.68	0.61	0.47	0.54	0.78	0.7		
6	422	0.46	0.56	0.34	0.47	0.41	0.61		
7	360	0.6	0.48	0.39	0.37	0.57	0.57		
8	342	0.48	0.66	0.37	0.49	0.57	0.62		
9	465	0.5	0.96			0.38	0.51	0.81	1.22
10	496	0.92	1.11			0.53	0.56	1.03	1.19

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